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EFFECTS OF TWO WEEKS DETRAINING ON METABOLIC FLEXIBILITY IN TRAINED OLDER ADULTS

Dissertação elaborada com vista à obtenção do Grau de Mestre em

Exercício e Saúde

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Abbreviations

ACSM	American College of Sports Medicine
AUC	area under the curve
BIA	bioelectrical impedance analysis
BMC	bone mineral content
BMI	body mass index
CV	coefficient of variation
DXA	dual-energy X-ray absorptiometry
FFA	free fat acids
FM	fat mass
FFM	free fat mass
MET	metabolic equivalent
MF	metabolic flexibility
MVPA	moderate-vigorous physical activity
PA	physical activity
RCT	randomized controlled trial
REE	resting energy expenditure
RDA	recommended dietary allowance
RMR	resting metabolic rate
RQ	respiratory quotient
TEF	thermic effect of food
VO_{2max}	maximal oxygen uptake
WHO	World Health Organization

Abstract

Background/Objective: Metabolic flexibility (MF) is highly influenced by lifestyle, in particular by physical activity and diet. However, little attention has been given to the impact of the adoption of sedentary behaviors, physical inactivity and detraining on MF especially in the older adults. Therefore, the aim of this study is to evaluate the effects of a two-week interruption of habitual supervised and structured exercise sessions on MF in trained older adults.

Methods: MF was evaluated in 7 older adults (3 females) aged ≥ 65 years (76.9 ± 6.5), before and after the short-term detraining, using indirect calorimetry to analyze the variance in the respiratory quotient (RQ) during the transition from fasting to 100 g oral glucose load. The test had a duration of 2 hours, and to measure the insulin response to the glucose load, blood samples were collected every 60 minutes (i.e. fasting, 60 min, and 120 min). To compare the difference between the moments, a general linear model for repeated measures was used adjusted for the covariates sex and age.

Results: There were only differences between the two moments in fasting RQ (P= 0.044). However, there was a trend for all the RQ and insulin mean values being higher after the detraining period. After detraining there was a decrease of 41.7% in RQ variance (P=0.231) and an almost threefold increase in insulin variance (P=0.692).

Conclusions: We showed that two weeks did not decrease MF among trained older adults. However, there is a trend to: (1) increase the reliance on carbohydrate as an energy substrate associated with a decrease in fat oxidation, (2) slow the response to the 100 g oral glucose load, (3) reduce RQ variance and increase that for insulin in response to the oral glucose load.

Key-words: physical inactivity, insulin, respiratory quotient, older adults, metabolic health, lifestyle, detraining, metabolic flexibility.

Resumo

Contextualização/Objetivos: A flexibilidade metabólica (FM) é influenciada pelo estilo de vida, nomeadamente pelo exercício físico e a alimentação. Porém, pouca atenção tem sido dada ao impacto do comportamento sedentário, da inatividade física e do destreino na flexibilidade metabólica, especialmente na população idosa. Assim, o objetivo deste estudo foi investigar o efeito de duas semanas de destreino na flexibilidade metabólica em pessoas idosas treinadas.

Métodos: A FM foi avaliada em 7 pessoas idosas (3 mulheres) com idade ≥ 65 anos (76.9 anos ± 6.5), antes e após as 2 semanas de destreino, utilizando a calorimetria indireta para analisar a variância no quociente respiratório (RQ) durante a transição do jejum para a ingestão de 100 g de glicose. O teste durou 2 horas, e para medir a resposta da insulina à carga de glicose, foi coletada uma amostra de sangue a cada 60 minutos (jejum, 60 min e 120 min). Para comparar a diferença entre os momentos foi utilizado um modelo linear geral para medidas repetidas, ajustado para as covariáveis sexo e idade.

Resultados: Apenas se verificaram diferenças entre os dois momentos no RQ em jejum (P=0.044). Contudo, é notória uma tendência para o aumento de todos os valores médios de RQ e insulina após o destreino. Com o destreino, verificou-se uma redução de 41,7% na variância do RQ (P=0,231) e um aumento quase triplo na de insulina (P=0,692).

Conclusão: Duas semanas de destreino não foram suficientes para diminuir a FM em pessoas idosas treinadas. No entanto, existe uma tendência para: (1) aumentar a utilização de carboidratos como substrato energético e diminuir a oxidação de gorduras, (2) diminuir a velocidade de resposta às 100 g de glucose, (3) reduzir a variância de RQ e aumentar a de insulina em resposta às 100 g de glicose.

Palavras chave: inatividade física, insulina, quociente respiratório, idosos, saúde metabólica, estilo de vida, destreino, flexibilidade metabólica.

Introduction

During the prehistory, human survival was dependent on the physical ability for hunting and, given the constant uncertainty about food availability, humans developed the capacity to have an extremely flexible metabolism that was able to supply energy needs through the use and conversion of another energy substrate as an alternative source of energy to guarantee an energy balance (Freese, Klement, Ruiz-Núñez, Schwarz, & Lötzerich, 2017).

With industrialization, access to food became almost instantaneous, requiring just a few steps to the kitchen, a phone call, or a short drive to the supermarket or a restaurant. Furthermore, technologic devices, occupation, and screen-based entertainment contribute to the reduction of physical activity (PA) and a substantial increase in sedentary time. Therefore, there was an increase in energy intake and a decrease in PA, making overfeeding and sedentary behaviors the new way of living (Bergouignan et al., 2010; Bergouignan, Rudwill, Simon, & Blanc, 2011; Chau et al., 2013).

Sedentary lifestyle and excessive eating habits contribute to the constant availability of energetic substrates that have given rise to many chronic disorders such as obesity and type 2 diabetes (Bergouignan et al., 2011; Rynders, Blanc, DeJong, Bessesen, & Bergouignan, 2017). It is also one of the causes for the inability to adapt substrate oxidation rates in response to changes in fuel availability, known as metabolic inflexibility (Rynders et al., 2017).

Older adults, besides going through processes that involve alterations in cardiovascular physiology and capacity, pulmonary function and respiratory capacity, neural function, endocrine functions, and body composition, are the most sedentary in the population (Harvey, Chastin, & Skelton, 2013; Judice, Silva, & Sardinha, 2015; Santos et al., 2018). These physiological alterations, cause deleterious effects on metabolism, making them less able to adapt the type of substrate being oxidized to the changes in fuel availability, which becomes

even more evident when coupled with an unhealthy lifestyle (Flack et al., 2010; López-Otín, 2013; McArdle et al., 2015).

Thus, it is possible to perceive that the adopted lifestyle has a high impact on the metabolism itself. In this regard, my dissertation will focus on the role of PA and sedentary behaviors on the regulation of metabolic flexibility (MF) in the older population.

After this theoretical framework that introduces the topic, emphasizing its relevance, the present dissertation will be composed by an extensive literature review that consists of a review of the current literature regarding MF definition, measurement techniques, determinant factors, the effects of lifestyle on MF, and its relation with aging. This section finishes by highlighting the pertinence of the study, with the main objectives and hypotheses outlined. Subsequently, the methodology will describe the population and the methods, followed by the results obtained, and the discussion. Lastly, the main conclusions of the present research along with future recommendations will be outlined.

Literature Review

1. Metabolic Flexibility

Metabolic flexibility (MF) is defined by the capacity to switch from predominantly lipid oxidation and high rates of fatty acid uptake during fasting conditions to the suppression of lipid oxidation and increased glucose uptake, oxidation, and storage after feeding (Kelley & Mandarino, 2000). The ability to adapt the fuel utilization to substrate availability is given by the respiratory quotient (RQ), defined as the variability of cellular rates of CO_2 production relative to O_2 consumption, measured by indirect calorimetry (Muoio, 2014).

During fasting, lipolytic pathways are increased whereas, in response to the transition from predominantly oxidative fatty acid metabolism to feeding, anabolic pathways are activated. This transition to anabolic pathways allows the substrate shift to efficiently utilize energy sources based on the content or mixture of the macronutrients in the meal (Galgani, Moro, & Ravussin, 2008; Goodpaster & Sparks, 2017). The primary purpose of the substrate shift is to move from catabolic to anabolic processes in order to store energy in skeletal muscle, adipose, and liver tissues (Goodpaster & Sparks, 2017).

A metabolically flexible individual is characterized by high variance in RQ, and low variance in plasma insulin concentration (Fig. 1-A), demonstrating a normal post-absorptive and postprandial adaptations in skeletal muscle substrate oxidation (Corpeleijn, Saris, & Blaak, 2009; Rynders et al., 2017). In contrast, a metabolically inflexible individual has a low variance in RQ for high variance in insulin (Fig. 1-B).

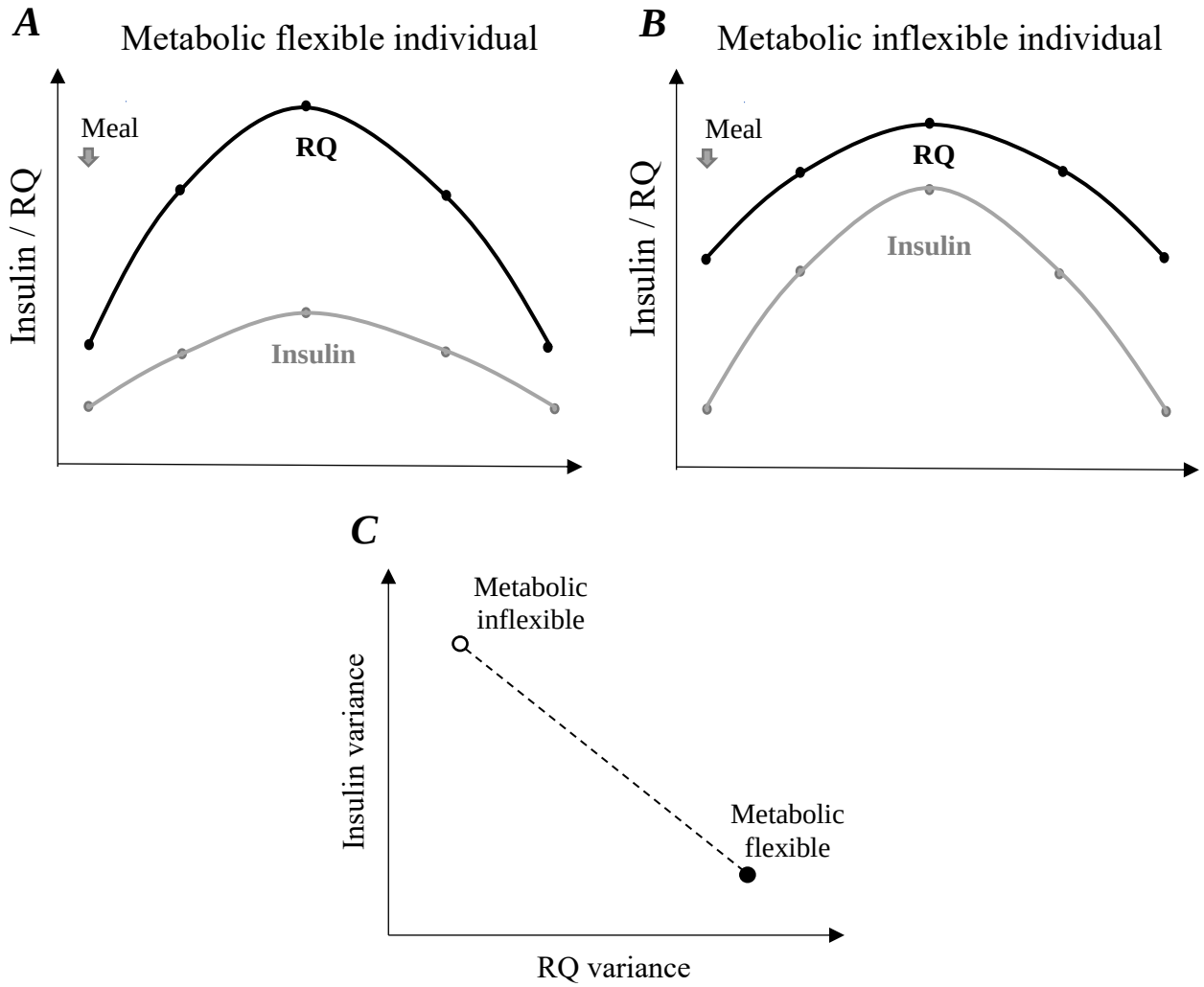

Fig. 1. The variance-based concept of metabolic flexibility.

Although MF is a relatively recent topic in the literature, it is known that a less flexible metabolism, is associated with several health risks and many pathologies. Given the relation of MF with insulin sensitivity, we can say that the large changes in insulin variance, which characterize an individual as metabolically inflexible, are related to insulin sensitivity impairment and/or insulin resistance. Therefore, a metabolic inflexible individual can have type 2 diabetes mellitus or are at greater risk of developing the disease. This metabolic inflexible condition is also associated with pathologies such as obesity, metabolic syndrome, cardiovascular disease, and cancer (Rynders et al., 2017; Smith, Soeters, Wust, & Houtkooper, 2018). Nevertheless, this deleterious effects on health can be prevented by a more flexible metabolism.

1.1. Measurement

The assessment of MF is, however, not clearly established. Numerous definitions of MF can be found in the literature as well as different ways of measuring it (Bergouignan et al., 2013). Albeit, MF is traditionally evaluated through the measurement of the RQ by indirect calorimetry during the last minutes of a hyperinsulinemic–euglycemic clamp or during the fasted to fed transition (Rynders et al., 2017).

Indirect calorimetry

Indirect calorimetry measures energy metabolism through the assumption that the heat produced as the food is oxidized in the body is directly proportional to the O_2 consumed and to CO_2 and H_2O produced in these reactions (Ferrannini, 1988). The amount of energy consumed over a period of time can be evaluated by the ratio between the amount of O2 consumed (VO_2) and the CO_2 produced (VCO_2), better known as the respiratory quotient (RQ) (McArdle, Katch, & Katch, 2015). Under a condition in which amino acids contribute to a minimum as an oxidative substrate, RQ can also provide information on the type and rate of substrate utilization (Ferrannini, 1988; Muoio, 2014).

The carbon and oxygen content in molecular formula of glucose and free fatty acids (FFA) are substantially different, which means that when oxidized, the amount of oxygen used differs according to the type of fuel (Kenney, Wilmore, Costill, & Osterberg, 2015). In general, the amount of oxygen required to fully oxidize a molecule of carbohydrate or fat is proportional to the amount of carbon in that fuel. Glucose molecule ($C_6H_{12}O_6$) contains six carbon atoms, meaning that during glucose oxidation six molecules of oxygen are used to produce six CO_2 molecules, six H_2O and 32 ATP molecules (Kenney et al., 2015). Thus, to oxidize carbohydrate the amount of O_2 required equals CO_2 produced and RQ is 1. On the other hand, FFA has considerably more carbon and hydrogen and less oxygen than glucose. So, even though fat

provides more energy than carbohydrate, in order to completely oxidize a molecule of fat, it is necessary to have a higher oxygen use and, the longer the chain of carbons this FFA contains, the more oxygen that will be needed to oxidize it (Kenney et al., 2015). Due to its higher consumption of O_2 compared to the production of CO_2, RQ value for fat is consequently lower than RQ for carbohydrate. Thus, without any carbohydrate contribution, RQ for fat is about 0.7 (Kenney et al., 2015).

Metabolic flexibility during a euglycemic-hyperinsulinemic clamp

The most common approach to evaluate the MF to carbohydrate is to measure RQ increase during a euglycemic-hyperinsulinemic clamp (Galgani, Moro, et al., 2008). This method is considered the gold-standard method to assess beta-cell and insulin sensitivity (J. Kim, 2009). It consists of the placement of two catheters, one placed in the arm to continuously infuse insulin and glucose and another in the contralateral hand for blood sampling (DeFronzo, Tobin, & Andres, 1979). The goal of the euglycemic insulin clamp is to raise, through insulin infusion, the plasma insulin concentration acutely to a new plateau and to maintain it at that level (DeFronzo et al., 1979).

To evaluate MF, resting metabolism and clamp-derived carbohydrate oxidation are needed. After an overnight fast, resting metabolism is conducted using indirect calorimetry to determine basal substrate oxidation (Malin et al., 2013). Clamp-derived carbohydrate oxidation is determined by indirect calorimetry during the last 20 minutes of the clamp (Malin et al., 2013). MF to glucose is calculated as the difference between the steady state RQ at the end of the clamp and fasting RQ (Galgani, Heilbronn, et al., 2008; Malin et al., 2013).

Metabolic flexibility to a meal

The measurement of MF using euglycemic-hyperinsulinemic clamp besides being an invasive and expensive method to assess insulin sensitivity does not reflect the physiologic responses of meal ingestion. Therefore, the measurement of the RQ by indirect calorimetry during fasting and following meal ingestion has been used as a less invasive and more realistic alternative to evaluate MF (Bergouignan et al., 2013; Heilbronn, Gregersen, Shirkhedkar, Hu, & Campbell, 2007).

Insulin and nonprotein RQ in response to a meal during indirect calorimetry are used to assess MF (Bergouignan et al., 2013). The relationship between the two variables metabolically characterizes the participant as flexible or inflexible (Bergouignan et al., 2013). RQ and insulin variance, area-under-the-curve (AUC), peak magnitude, time-to-peak-magnitude, and slope of initial rise are used in order to evaluate intraindividual responses to a meal (Calçada et al., 2014).

2. Effects of lifestyle

The regulation of fuel consumption and energy demand that affects MF requires an interaction of extrinsic and intrinsic metabolic regulators and effectors (Rynders et al., 2017). The intrinsic and extrinsic factors that contribute to the incapacity to switch the type of substrate being oxidized (carbohydrate and fat oxidation) in response to changes in fuel availability are not well understood. However, it is known that they depend on the mutual influence of lifestyle factors (diet, PA, others) and physiological factors (Corpeleijn et al., 2009; Rynders et al., 2017). This topic will address the influence of lifestyle and how it affects the MF.

2.1. Diet

Since it provides energy that preserves the structural and functional integrity of the organism, diet, and energy balance play a key role in human metabolism (McArdle et al., 2015). This section comprises protein, carbohydrate, and lipid recommendations, physiological functions, and association with MF. Energy balance, its importance for weight maintenance and the role of a balanced diet in MF will also be addressed.

2.1.1. Protein

Protein is considered the most important and versatile macronutrient, due to its engagement in every biological process within the body (Wolinsky & Driskell, 2008). About 15% of body weight is attributed to protein, almost half of this protein (43%) is in the skeletal muscle and 10% in metabolically active visceral tissues (liver and kidney). Structural tissues such as skin and blood contain nearly 15% of total protein each, with other organs such as brain, lung, heart, and bones contributing to the remainder (Institute of Medicine, 2005; Lentner, 1981).

The human body is completely dependent on the protein obtained through food to maintain essential body function, protein levels, protein synthesis and amino acid needs (Wolinsky & Driskell, 2008). Dietary protein can come from either animal or vegetal sources. Animal proteins normally contain the nine essential amino acids that we only have access through food and for this reason, are named as complete proteins or high-quality proteins (McArdle et al., 2015). Whereas plant-derived protein, except soybeans that contain all essential amino acids, remain incomplete in one or more essential amino acids and is considered an incomplete protein or with lower biological value (McArdle et al., 2015).

Recommended protein intake

Currently, the Recommended Dietary Allowance (RDA) for protein is 0.8g protein/kg of body weight/day, for all adults, regardless of age or sex. In turn, older adults (+65), taking into account the many physiological changes that occur with aging, require a higher protein consumption of 1.0–1.6 g per kg of body weight/day, in order to support good health, promote recovery from illnesses, and maintain functionality (Bernstein, Munoz, Academy of, & Dietetics, 2012; Deer & Volpi, 2015; Deutz et al., 2014).

Role of protein in the body

Protein is a macronutrient that provides 4kcal per gram, with mainly structural and metabolic functions, although under some circumstances, may contribute to energy metabolism (Wolinsky & Driskell, 2008). Protein does not have a store, unlike carbohydrates and lipids, whose primary roles are for energy use. All protein contributes to tissue structures or exists as important constituents of metabolic, transport, and hormonal systems (McArdle et al., 2015; Wolinsky & Driskell, 2008). Just in some situations, protein in amino acid form has a prominent role in energy metabolism and may be converted in FFA, pyruvate, glycogen, acetyl units or glucose and used as an energy substrate (Wolinsky & Driskell, 2008).

Protein and metabolic flexibility

Protein oversupply does not influence total body protein. Its increase is exclusively dependent on growth stimuli, such as growth hormone, androgens, physical training and weight gain (Galgani & Ravussin, 2008, 2009).

Unlike carbohydrates and lipids, whose primary roles are for energy use, protein does not have a store, all protein contribute to tissue structures or exist as important constituents of metabolic, transport, and hormonal systems (McArdle et al., 2015; Wolinsky & Driskell, 2008).

Consequently, its use as energy results in functional and structural protein loss, primarily from skeletal muscle and then from the remaining organs (McArdle et al., 2015).

For these reasons, protein consumption and imbalance cannot be implicated as a direct cause of obesity neither as a factor of MF (Galgani & Ravussin, 2008).

2.1.2. Carbohydrates

Carbohydrate is a macronutrient available on leaves, vegetables, fruits and grains that name derives from its chemical composition constituted by atoms of carbon, hydrogen, and oxygen (McArdle et al., 2015). As a protein, a gram of carbohydrate provides 4kcal. It is an essential macronutrient for metabolism in many tissues and organs, with the primary role of providing energy to body cells. It is the most important source of energy during high-intensity exercise since it is practically the only source utilized during maximal and supramaximal exercise (Wolinsky & Driskell, 2008). Furthermore, brain, retina, and red blood cells are totally dependent on glucose for energy, however, during carbohydrate depletion, larger amounts of fat are metabolized and used as ketones for fuel (Wolinsky & Driskell, 2008). The RDA for carbohydrate takes into account the minimum amount of glucose required by the brain without depending on alternative energy sources, resulting in a minimum consumption of 130 g/d for men and women starting at 1 year old. However, the RDA is increased in pregnant and lactating women (Trumbo, Schlicker, Yates, & Poos, 2002).

Carbohydrate and metabolic flexibility

Despite being the macronutrient that is usually the main source of dietary energy, the quantity of energy stored in carbohydrate is very limited, and consist of glycogen stores from liver, kidney, muscle, and other tissues plus the glucose that circulates in the blood (Bray, 1991).

When carbohydrates are consumed blood glucose levels, as glycogen storage and glucose oxidation all increase, while fat oxidation is suppressed, leading to a rise in RQ (Galgani, Moro, et al., 2008; Wolinsky & Driskell, 2008). Under normal conditions carbohydrates consumed are not stored efficiently due to the constant need for energy by some tissues and its primary use for energy supply. Although, when carbohydrate intake is significantly greater than its oxidation and glycogen stores are full, carbohydrates are stored as fat in the adipose tissue through lipogenesis (Wolinsky & Driskell, 2008). A positive energy balance and an excessive amount of carbohydrates are required for fat accumulation in adipose tissue and weight gain (Galgani & Ravussin, 2008, 2009). In a healthy 70 kg human, muscle glycogen storage contains approximately 400 g and can only be used by the muscle itself. Liver glycogen, in turn, contains around 100 g and is available to the whole organism through the bloodstream (McArdle et al., 2015; Wasserman, 2009). Given the fact that the body storage system for carbohydrates as glycogen is minimal, excess calories in the form of lipids or protein do not increase glycogen stores (Bray, 1991; Stubbs, Ferres, & Horgan, 2000).

Therefore, since the energy balance must be considered, excess carbohydrate intake alone cannot be the basis of weight gain or considered as a key factor for impairment in MF (Galgani & Ravussin, 2008; Wolinsky & Driskell, 2008).

2.1.3. Lipids

Lipids have important functions in the body including thermal insulation, vitamin carrier, hunger suppression, structural components of cells, protection of vital organs, and as an energy source (McArdle et al., 2015). It provides more than double the energy of carbohydrates and protein, being the most caloric energy source with 9 kcal per gram. It is also the main energy supplier under low-calorie dieting or fasting conditions, cold stress, low to

moderate intensity exercise, and prolonged exercise that depletes glycogen reserves (McArdle et al., 2015).

The recommendations for total fat are formulated in order to prevent the fall in HDL cholesterol, with a lower limit no less than 20% and upper range of 35% of total energy intake (Trumbo et al., 2002).

Lipid and metabolic flexibility

Contrarily to protein and carbohydrate, fat storage is unlimitedly reaching between 60000 and 100000 kcal in a typical young male adult whereas carbohydrate energy reserves generally are less than 2000 kcal (McArdle et al., 2015). While under normal conditions, the excess of carbohydrates will not be stored as fat, any dietary fat included in the meal that is not utilized for energy will be stored in the adipose tissue, and body fat gain can result (Wolinsky & Driskell, 2008). Under positive energy balance, the energy consumed in excess can result in fat storage due to the efficiency of storing dietary fat over other fuels (Wolinsky & Driskell, 2008).

Accordingly, considering energy balance in humans under physiological conditions, fat is the only nutrient capable of causing a chronic imbalance between intake and oxidation, directly contributing to the increase in adipose tissue (Galgani & Ravussin, 2008). Thus, increased dietary fat may affect the capacity of the body or cells to match fuel oxidation to fuel availability and consequently affect MF (Galgani & Ravussin, 2008).

2.1.4. Energy balance

Changes in the day to day energy and macronutrient intake and daily variation in energy expenditure lead to either positive or negative energy balance (Galgani, Moro, et al., 2008). Nevertheless, not only the energy intake and energy expenditure must be taken into account

but also macronutrient intake and oxidation. Consequently, in order to maintain weight and do not store or lose energy stored, energy intake has to match energy expenditure, as well as macronutrient intake must balance macronutrient oxidation (Galgani, Moro, et al., 2008; Galgani & Ravussin, 2008).

Under positive energy balance, the mitochondria are overwhelmed by an excess in substrates derived from fatty acids, glucose, and amino acids. This surplus leads to mitochondrial metabolic 'indecision' and ineffective substrate switching, which results in higher energy consumption/expenditure ratio and subsequent storage of substrates (Smith et al., 2018). This storage can be either glycogen from carbohydrates or triglycerides from any macronutrient (Dunstan, Healy, Sugiyama, & Owen, 2010).

Under normal feeding conditions, as well as carbohydrates, increased protein is also followed by a rise in protein oxidation (Wolinsky & Driskell, 2008). Fat intake increase, in turn, does not immediately increases fat oxidation proportionately (Wolinsky & Driskell, 2008). Thereby, the varied responses of carbohydrate, protein, and fat oxidation to changes in macronutrients intake emphasize the importance of nutrient composition in energy balance (Wolinsky & Driskell, 2008).

2.1.5. Macronutrient balance

The ratio of fat and carbohydrate in the diet is the primary factor in the macronutrient composition of the diet that easily causes a positive energy balance that leads to weight gain (Saris, 2003). In this subtopic, the importance of a balanced diet and how diet composition can affect energy balance and MF will be addressed.

Low fat vs low carbohydrate diet evidence from systematic reviews

As mentioned before, carbohydrate consumption suppresses fat utilization and oxidation, so low carbohydrate diets promote the use of fat as the main energy source, and due to carbohydrate depletion larger amounts of fat will be metabolized and used as ketones for fuel by the organs totally dependent on glucose (Wolinsky & Driskell, 2008). However, despite the larger amount of fat metabolized when carbohydrate is depleted, fat remains the most caloric energy source, providing more than double the energy produced by carbohydrates and protein with 9 kcal per gram. Besides, fat is also the macronutrient that has the least energy expenditure in its processing, affecting less than 5% of the total thermic effect of food (McArdle et al., 2015). Thereby, since each gram of carbohydrate contains only 4 kcal and one gram of fat 9 kcal, we can consume more than twice the grams of carbohydrates as of lipids for the same number of kilocalories.

With respect to the satiating effects of the macronutrients, the protein appears to be the most satiating, followed by carbohydrate, and lastly fat (Stubbs et al., 2000). Consequently, high-fat foods can readily stimulate high intake of fat energy with no proportionate increase in satiating power (Blundell, Lawton, Cotton, & Macdiarmid, 1996). Furthermore, high-fat diets tend to be associated with insulin resistance while high carbohydrate diets are generally associated with improvements in insulin sensitivity in the short-term (Wilcox, 2005). In addition, the brain's dependence on carbohydrates as an energy source make the ketogenic low-carbohydrate diets cause tiredness and a greater sensation of hunger, being more difficult to adhere to in the long-term (Hu & Bazzano, 2014).

In summary, although both diets are effective for weight loss, carbohydrates consumption becomes essential to maintain the diet in the long term. The consumption of a balanced diet is, therefore, crucial for the maintenance of weight and to have a flexible

metabolism, both in energy level and in macronutrient distribution according, and intake according to the RDA.

2.1.6. Energy expenditure

Resting energy expenditure (REE)

This component reflects the minimum amount of energy required to sustain vital physiologic functions in the waking state. Plus, it is the one that contributes most to the total energy expenditure from 50% to 70%, depending on daily activities and physical exercise (Kenney et al., 2015; McArdle et al., 2015). REE is influenced by a number of factors. Body size, which includes both body mass and body area, account for about half of the variation in basal metabolism, meaning that an increase in body size results in a boost of basal metabolism (McArdle et al., 2015). Body composition and sex are also determinant factors. Since fat tissue has lower metabolic activity than muscle and women normally possess more body fat and less fat-free mass than men of similar size, women have lower energy expenditure than men (McArdle et al., 2015). Age is another extremely influential factor due to the energy cost of growth in children and young adults under the age of 20, that leads to a higher metabolic value (Levine, 2004; Levine et al., 2005; McArdle et al., 2015). Weather, body temperature, ethnicity, health, fitness status, hormonal status, smoking habits, and consumption of stimulant substances such as caffeine are factors that have also have an impact (McArdle et al., 2015).

Thermic effect of food (TEF)

Diet-induced thermogenesis represents the energy consumed in digestion, absorption, transport, metabolism, and storage of food. Thereby, food consumption generally increases energy metabolism and represents about 10% to 15% of the total daily energy expenditure (Levine, 2004; McArdle et al., 2015). The way the food is ingested, the quantity and type of

food consumed, the fractionation of the meals during the day, food temperature, and caffeine content affect energy metabolism (Stubbs et al., 2000). The type of macronutrients consumed also have a different impact on TEF (McArdle et al., 2015). Fat besides being the most caloric energy source with 9kcal per gram is also the macronutrient that has the least energy expenditure in its processing, affecting less than 5% of the total value of TEF (McArdle et al., 2015; Stubbs et al., 2000). Although proteins and carbohydrates both provide 4kcal per gram, protein has a major thermogenic value that can reach 30%, while carbohydrates contribute from 5% to 10% of TEF (McArdle et al., 2015; Owen et al., 2011; Tappy, 1996).

Activity-induced energy expenditure

Represents the thermic effect of any movement beyond basal metabolic rate and consists of exercise-related activity thermogenesis (planned, structured, and repetitive, which have an objective to improve or maintain one or more components of physical fitness) and non-exercise activity thermogenesis (includes the energy expenditure of occupation, leisure, sitting, standing, walking, talking, playing instruments, dancing, house cleaning, shopping, among others) (Levine, 2004). Due to the significant variations of the type of behavior adopted in a daily basis, oscillating between predominantly sedentary behaviors or quite active, it is the most variable component of energy expenditure within and between subjects contributing from 15% to 50% or more of total energy expenditure (Levine, 2004; Levine et al., 2005).

2.2. Sedentary behavior

Today's environment has been influenced by the emergence of industrialization and technology evolution. Longer distances between places lead to a decline in walking and cycling due to the increase in car use and traffic. Labour-saving machines, poor neighbourhood facilities, recreation environment promoting sitting parks, technological devices and screen-

based entertainment (cinema, videogames, television) also contribute to an increase in sedentary time and consequently, a decrease in activity-induced energy expenditure (Chau et al., 2013; Lanningham-Foster, Nysse, & Levine, 2003; Thorp, Owen, Neuhaus, & Dunstan, 2011).

Sedentary behavior is defined by any waking behavior with relatively low energy expenditure, inferring a lack or absence of muscular contraction ≤1.5 MET while in a sitting or reclining posture (Dempsey, Owen, Biddle, & Dunstan, 2014; Tremblay et al., 2017). Time in sedentary behavior can be spent in domestic environments and leisure time (TV viewing and other recreational screen time), jobs that require prolonged sitting (screen-based jobs) and in transportation vehicles (Owen et al., 2011).

2.2.1. Epidemiology

It is estimated that people around the world spent, in average, 6.4 hours per day sitting in a range from 3.8 to 11.9 hours (Bauman, Petersen, Blond, Rangul, & Hardy, 2018). When stratifying to objectively measured methods that use motion sensing devices, such as accelerometers and inclinometers, to calculate precisely sedentary time, total sitting time increase 2 hours, with an average of 8.2 hours per day of sedentary behavior (Bauman et al., 2018). Studies also showed that, among adults, time spent sitting increased with age and higher education, and high rates of work-related sitting time. Contrarily, the time spent on television watching is higher among lower socio-economic groups and among older adults (Bauman et al., 2018).

Few studies have been conducted to measure sedentary behavior in older adults. However, a systematic review reported that, when objectively measured, 67% of the older population around the world were sedentary more than 8.5 hours per day (Harvey et al., 2013). Those go in accordance with The U.S. NHANES survey (Evenson, Buchner, & Morland, 2012)

and the Canadian Health Measures Survey (Copeland, Clarke, & Dogra, 2015) findings, which showed that older people spend 8.5 to 10 hours per day, respectively, on sedentary behavior.

Among 20 countries and according to self-reported data, Portugal manifest the lowest prevalence of sitting time, 76% of its total population reported spending less than 4 hours seated (Bauman et al., 2011). However, self-reported measures are less accurate, leading to an overestimation error. Thus, when measured with accelerometers, Portuguese adults total sitting time increased 5 hours, resulting in an alarming 9 hours of sedentary behavior (Baptista et al., 2012; Judice et al., 2015; Santos et al., 2018). Older adults are the most sedentary group in the population, and this is not an exception in Portugal. Portuguese elderly men spend over 10 hours seated, on average, which is 20 - 30 minutes more than an elderly woman (Baptista et al., 2012; Judice et al., 2015).

2.2.2. Sedentary behavior *vs* Physical Inactivity

The term 'sedentary' can sometimes, be mistaken with the term "inactive", which is used to describe those who are performing insufficient amounts of PA defined by ACSM and AHA (Dempsey et al., 2014; Sedentary Behaviour Research, 2012). Nevertheless, sedentary lifestyle and PA can co-exist, a physically active individual, who fulfill the recommendations defined by ACSM, can also spend most of the day in sedentary behavior (Owen, Healy, Matthews, & Dunstan, 2010). Another pattern, less common, is a non-sedentary individual who barely spends time sitting, however, performs insufficient amounts of moderate and vigorous PA (MVPA) (Dempsey et al., 2014). Although MVPA appears to attenuate the risks of sedentary behavior, unrealistic levels of MVPA appear to be needed to completely eliminate the risks of large volumes of sedentary behavior (van der Ploeg & Hillsdon, 2017). In addition, since time spent in sustained MVPA represented only a small fraction of their waking time each day (± 2%), and individuals can spend the majority of their waking day sitting (± 63% of

their waking hours), variations in MVPA are not significantly associated with sitting or intermittent stepping time (Chau et al., 2013; Hamilton, Hamilton, & Zderic, 2014). For that reason, time spent sitting is distinct and not related to time spent performing sustained MVPA and regular exercisers are not necessarily less sedentary (Chau et al., 2013; Hamilton et al., 2014).

Therefore, the ideal behavior pattern for health outcomes is to fulfill the MVPA recommendations and lower sedentary time by often including low-intensity movements like standing and light walking to interrupt sedentary behavior, on a daily basis (Dempsey et al., 2014).

2.2.3. Generical effects on health

Despite the deleterious effects, daily sitting time seems to be attenuated in the presence of MVPA. There is building evidence showing that the chronic, unbroken periods of muscular inactivity associated with prolonged sitting time have consequences on several metabolic health outcomes (Chau et al., 2013; Katzmarzyk, Church, Craig, & Bouchard, 2009; van Uffelen et al., 2010). In this topic, the harms of sedentary behavior will be stratified according to the type of studies and research designs.

Evidence from observational studies

Three large prospective cohort studies with a follow up of 1.5, 8.5 and 9.5 years, reported that independent of age, sex, body mass index (BMI), education level, smoking status, alcohol consumption, total PA energy expenditure, medication, diabetes history, family history of CVD, and cancer, each additional hour of daily sitting time was associated with an increase in all-cause mortality, cardiovascular-related mortality, and other causes but not with cancer (Katzmarzyk et al., 2009; Matthews et al., 2012; Wijndaele et al., 2011). Even among

individuals reporting high levels of MVPA (more than seven hours per week), seven hours per day of sedentary behavior was associated with 50% greater risk of all-cause mortality and a twofold greater risk of cardiovascular mortality (Matthews et al., 2012).

A cross-sectional study used an accelerometer for 7 days in 169 adults to monitor the associations between the percentage of time spent sedentary, in light-intensity, and in MVPA with waist circumference, triglycerides, HDL cholesterol, resting blood pressure, fasting plasma glucose, and clustered metabolic risk score (Healy, Wijndaele, et al., 2008). These authors found that the cumulation of sedentary time and the manner in which it is accumulated may also be relevant for these health outcomes. While time spent sitting is related with metabolic risk, breaking up sedentary time is inversely associated with unhealthy outcomes and metabolic risk markers (Healy, Dunstan, et al., 2008; Healy, Wijndaele, et al., 2008; Judice et al., 2015).

Evidence from randomized controlled trial (RCT) studies

Most of the RCT studies on sedentarism were conducted to understand the effects of interrupting sitting time, with rarely or almost no studies in which the intervention consisted in an increase in sedentary behavior and/or a decrease in PA.

A recent experimental study fulfilled these requirements by performing an intervention with a short-term (14 days) reduction in PA and increased sedentary behavior, from a mean daily step count of >10,000 to 1500 steps a day. Their findings demonstrated a reversible reduction in multi-organ insulin sensitivity and cardiorespiratory fitness, with concomitant increases in central and liver fat and dyslipidemia (Bowden Davies et al., 2018). In older adults, two weeks of step reduction (daily steps ≤ 1000 steps) led to lowered rates of muscle protein synthesis and impaired glycaemic control that, unlike younger adults, was not recovered during the following 2 weeks of return to regular activity (McGlory et al., 2018).

Interventions breaking up sedentary behavior reported that increasing the short bouts of standing, light or moderate intensity walking lowers postprandial glucose, insulin levels, increased energy expenditure, and fat oxidation over an 8 h postprandial observation period (Dunstan et al., 2012; Hawari, Al-Shayji, Wilson, & Gill, 2016; Henson et al., 2016). However, compared with low-intensity exercise, moderate intensity exercise is more effective in reducing postprandial plasma triglycerides (I. Kim, Park, Trombold, & Coyle, 2014). This indicates that the frequency of interruptions, probably due to the increased energy expended with muscular contractions in the sit-to-stand and stand-to sit transitions, has a marked independent influence on metabolic rate (Hawari et al., 2016; Judice, Hamilton, Sardinha, Zderic, & Silva, 2016).

Evidence from the systematic reviews and meta-analysis studies

A considerable number of systematic reviews looking into observational studies indicate that sedentary time, independent of PA, is associated with an increased risk of weight gain, insulin resistance, cardiovascular disease, and all-cause mortality (Chau et al., 2013; Patterson et al., 2018; Thorp et al., 2011; Wilmot et al., 2012).

A threshold of 6 to 8 h/day of total sitting and 3 to 4 h/day of TV viewing, above which the risk for all-cause mortality is increased, were identified (Patterson et al., 2018). Due to its link to higher intakes of energy and macronutrients along with greater energy from snacks, TV viewing is proposed as the most deleterious sedentary behavior (Owen et al., 2010; Patterson et al., 2018).

Regardless of total sedentary time and the time spent in PA, people who interrupted their sedentary time more frequently (breakers) had a better metabolic profile than those whose sitting time was mostly uninterrupted (prolongers) (Benatti & Ried-Larsen, 2015; Groeneveld, Proper, van der Beek, Hildebrandt, & van Mechelen, 2010; Owen et al., 2010).

It was evaluated that 5.9% of deaths could be attributed to daily total sitting time, even with PA taken into account (Chau et al., 2013; Patterson et al., 2018). This value is similar to other major risk factors reported by the World Health Organization such as tobacco use (8.7%), physical inactivity (5.5%), and overweight and obesity (4.8%). It suggests that if daily sitting time would be reduced, the beneficial effect on population health could be comparable to that achieved by reducing smoking, inactivity, or obesity (Chau et al., 2013). Therefore, considering the adverse health outcomes, sedentary behavior is indicated as a distinct and independent health risk factor.

2.3. Physical inactivity and detraining

Exercise, despite being used interchangeably with PA, is a subcategory of it that assumes planned, structured and repetitive PA, which have a goal to improve or maintain one or more components of physical fitness (Caspersen, Powell, & Christenson, 1985). To improve health, lower susceptibility to disease (morbidity), and decrease premature mortality, ACSM/AHA recommends a weekly accumulation of at least 150 min of moderate-intensity aerobic activity (3.0 to 5.9 MET's), 75 min of vigorous PA (≥6.0 MET's) or a combination of both (American College of Sports, Riebe, Ehrman, Liguori, & Magal, 2018). Additionally, regular PA is one of the main determinants of energy expenditure and is therefore fundamental to energy balance, weight control and obesity prevention (World Health Organization, 2014). In contrast, as mentioned above, the term physically inactive is used to describe those who are performing insufficient amounts of PA defined by the ACSM, while detraining is the partial or complete loss of training-induced anatomical, physiological, and performance adaptations as a consequence of training reduction or cessation (Dempsey et al., 2014; Mujika & Padilla, 2000).

2.3.1. Epidemiology

Insufficient PA is one of the 10 leading risk factors for global mortality, responsible for 3.2 million deaths each year (Lim et al., 2012). Compared with those who comply with PA recommendations, insufficiently active adults have a 20−30% increased risk of all-cause mortality (Lee et al., 2012). Worldwide, is estimated that physical inactivity is responsible for 6% of the burden of disease from coronary heart disease, 7% of type 2 diabetes, 10% of breast cancer, and 10% of colon cancer (Lee et al., 2012).

The WHO reported that in 2010, women worldwide were less active than men, about 27% of women versus 20% of men did not reach the recommended level of activity. Insufficient PA in adults increased according to the level of country income and age, with 19% of the youngest age group not meeting the recommendations, compared to 55% of the oldest age group (World Health Organization, 2014).

According to the Eurobarometer survey on sport and PA, based on about 68% of the population, Portugal is one of the three countries most likely to never exercise or play sport in Europe. Just as reported by the WHO, Portuguese women are less active than men, 78% versus 68% and the levels of PA also decrease with age (European Commission, 2017). Considering that the ideal behavior pattern for health outcomes is low levels of sedentary time, and fulfillment of PA recommendations, Portugal's alarming levels of physical inactivity and sedentarism can lead to adverse health and economic burden.

2.3.2. Effects of short-term detraining

The changes caused by training cessation or insufficient training differ according to their duration. In this way, detraining is divided by short-term when it has less than 4 weeks of insufficient training stimulus and long-term when lasts more than 4 weeks (Mujika & Padilla,

2000). Given the short duration of the detraining in the present investigation, this section will focus on the consequences of short-term detraining.

Effects on cardiorespiratory capacity

Maximal oxygen uptake (VO_{2max}), although suffers greater changes in the long term, has been shown to decline with short term detraining, depending on the physical activities performed during that time (Ehsani & Spina, 1997; Mujika & Padilla, 2000). There are several references that reported a decrease in VO_{2max} between 4 and 14% with short term detraining (Coyle et al., 1984; García-Pallarés, Carrasco, Díaz, & Sánchez-Medina, 2009; Houmard et al., 1992; Houmard et al., 1993), with this change ever more noticeable in older adults (Lobo, Carvalho, & Santos, 2010; Toraman, 2005). In the short term detraining, as a result of a loss in both red cell volume and plasma volume (Mujika & Padilla, 2000), blood volume decreases by 5 to 12% (Ehsani & Spina, 1997; García-Pallarés et al., 2009; Houmard et al., 1992). This blood volume reduction makes heart rate increase about 5% to 10% at submaximal and maximal intensities and, when combined with the decrease in left ventricular wall thickness induced by detraining, reduces stroke volume up to 17% (Coyle, Hemmert, & Coggan, 1986; Martin, Coyle, Bloomfield, & Ehsani, 1986; Pedlar et al., 2018). Cardiac output, in turn, is the product between heart rate and stroke volume, and despite the rise in heart rate values resulting from cardiovascular detraining, it does not seem to be enough to counterbalance the reduction in stroke volume, causing a reduction of cardiac output (García-Pallarés et al., 2009). It was also shown a rapid deterioration of ventilatory function characterized by a decline in maximal ventilatory volume, which often declines in parallel with VO_{2max} and O_2 pulse - the amount of oxygen consumed in ml/systole (Ghosh, Paliwal, Sam, & Ahuja, 1987; Mujika & Padilla, 2000).

Muscular effects

Capillary density, as VO_{2max}, is more affected by long term detraining and, in the short term, its reductions are small or even non-existent (Mujika & Padilla, 2000). Muscle fiber distribution in resistance athletes is also not affected in the short term (Neufer, Costill, Fielding, Flynn, & Kirwan, 1987). However, muscle cross-sectional area derived from muscle hypertrophy, declines in strength and speed athletes, due to the reduction in type 2 fibers area and also by the neural and muscular adaptations caused by the inactivity (Häkkinen & Komi, 1983; Staron et al., 1991). There is a trend for a more significant difference in strength after detraining in older adults (Hakkinen, Alen, Kallinen, Newton, & Kraemer, 2000; Kalapotharakos, Diamantopoulos, & Tokmakidis, 2010).

Mitochondrial ATP production and muscle glycogen concentration, in turn, are variables that reduce significantly in the short term (Wibom et al., 1992). In isometric and isokinetic concentric strength, there are no differences on the bench press, squat, and leg extension in short term detraining (Hortobagyi et al., 1993). On the other hand, electromyogram activity and isokinetic eccentric strength are reported to decline at a much higher rate (Hortobagyi et al., 1993).

Metabolic effects

At the metabolic level, short periods of insufficient training are associated with increased RQ at submaximal and maximal exercise intensities, which indicates a higher reliance on carbohydrate as a substrate for exercising muscles (Mujika & Padilla, 2000, 2001). Despite no difference in resting and fasting RQ with detraining, a seven-day bed rest study showed a significant increase in fasting RQ that manifest lower lipid oxidation (Blanc et al., 2000). Inactivity decreases rapidly sensitivity for insulin-mediated whole-body glucose uptake (Mujika & Padilla, 2000; Vukovich et al., 1996). This decline may be associated with a reduced

muscle GLUT4 transporter protein content, which has been shown to decrease by 17 to 33% after 6 to 10 days (Mujika & Padilla, 2000; Vukovich et al., 1996). On the other hand, short-term training cessation from endurance training has been shown to yield a condition that favors the storage of adipose tissue, due to a marked increase in adipose tissue lipoprotein lipase activity, coupled with a marked decrease in muscle lipoprotein lipase activity (Mujika & Padilla, 2000, 2001).

2.4. Sedentary behavior, physical inactivity, detraining, and metabolic flexibility

As mentioned previously, physical inactivity and sedentary behavior have several adverse health effects and should be considered as independent risk factors. But, although their risks are independent of each other, the mechanisms by which they are related to metabolic impairment and consequently to metabolic inflexibility are similar. Some bed-rest, increased sedentary behavior, reduced PA, and detraining studies have been conducted to evaluate their effects on metabolic profiles.

An RCT with forty-five habitually active participants demonstrate that a 14-day reduction in PA with increased sedentary behavior leads to a reversible reduction in multi-organ insulin sensitivity and cardiorespiratory fitness, with concomitant increases in central and liver fat and dyslipidemia (Bowden Davies et al., 2018). Another RCT with 44 participants showed that both a reduction in spontaneous and structured PA for one month decreased the variance in daily RQ and increased that of insulin, which suggests a decrease in MF (Bergouignan et al., 2013). This is consistent with the findings from a systematic review from bed-rest studies which reported that the variances of insulin and RQ are inversely but linearly associated along a PA continuum (Bergouignan et al., 2011).

In conclusion, regardless of energy balance conditions, the reduction of PA levels along with increased sedentary behavior can lead to muscle atrophy due to the decreased mechanical

workload. An increased reliance on carbohydrate as an energy substrate with a decrease in fat oxidation and an accumulation of lipid in ectopic tissues associated with an increase in plasma insulin concentration or a decrease in insulin action may also be a consequence of a reduction of PA (Bergouignan et al., 2013; Corpeleijn et al., 2009; Stein & Wade, 2005). These may be the explanatory metabolic mechanisms induced by prolonged inactive periods that explain how sedentary behavior and physical inactivity can induce metabolic inflexibility.

3. Determinant factors of metabolic flexibility

After explaining the impact of lifestyle on MF, we will address the intrinsic physiological factors related to it, which include insulin sensitivity, the capacity to store and release FFA from adipose tissue, mitochondrial oxidative capacity, and skeletal muscle fiber type.

3.1. Insulin

Insulin is an anabolic hormone secreted by the beta-cells of the pancreas driven by the rising of blood glucose after a carbohydrate-rich meal (Czech, 2017; Storlien, Oakes, & Kelley, 2004). Insulin secretion has the purpose of maintaining normal blood glucose levels by facilitating cellular uptake into muscle and adipose tissue through insulin sensitive regulator glucose transporter protein 4 (GLUT4), boosting glycolysis and glycogen synthesis (Czech, 2017; Wilcox, 2005). It is the main hormone that regulates cellular energy supply, macronutrient balance, and directs anabolic processes during fed condition (Wilcox, 2005).

Insulin, glucose disposal rate, and metabolic flexibility

Glucose is the main stimulus for insulin secretion, although other factors as macronutrients, hormones, humoral factors, and neural input may alter its response (Wilcox,

2005). Considering its role as intra-cellular transporter of glucose into insulin-dependent tissue, such as muscle and adipose tissue, insulin release after a meal is the main driver of substrate utilization change from predominantly oxidative fatty acid metabolism to glucose oxidation in the skeletal muscle (Goodpaster & Sparks, 2017; Wilcox, 2005).

During fasting, insulin and glucose levels are low, and muscles rely on fatty acids and amino acids utilization as their main energy source. When a meal is consumed, exogenous energy is provided through the macronutrients contained in foods, causing adipose tissue fat breakdown suppression and its synthesis promotion (Wilcox, 2005). The change from fasting to feeding allows glucose entry to muscle cells causing glycogen to be synthesized and stored, which enables the use of carbohydrates as the main source of immediately available energy, instead of fatty acids or amino acids (Wilcox, 2005). In the fed state, insulin promotes glycogen and lipid synthesis in muscle cells and suppress lipolysis and gluconeogenesis from muscle amino acids (Wilcox, 2005). This allows the use of glucose or glycogen as energy to be released via glycolysis during a glycolytic or oxidative muscular activity (Wilcox, 2005). Therefore, insulin, due to its function of glucose uptake, plays a key role in glucose metabolism.

Small changes in insulin in response to a meal are associated with a metabolically flexible state, whereas a metabolically inflexible person is characterized by a low amplitude in RQ marked by a large variance in insulin, with the variance in plasma insulin concentration has a major influence on MF (Rynders et al., 2017).

These large changes in insulin variance, which characterize an individual as metabolically inflexible, are related to insulin sensitivity impairment, better described as insulin resistance. Individuals are characterized as insulin resistant when they have type 2 diabetes or they are at risk of developing the disease. Insulin resistance is defined as a decrease of the target cell's metabolic response to the hormone, at the whole-body level, an impaired sensitivity to insulin in which cellular glucose uptake is decreased and cellular glucose

available for oxidation is low (Wilcox, 2005). This leads to an untypical rise in blood glucose levels, thus increasing insulin secretion by beta-cells (Czech, 2017). In insulin-resistant individuals, impaired glucose storage is expected, since increased insulin secretion enhances blood insulin concentration, causing large variance in postprandial insulin levels that along with a slight variance in RQ typically describe metabolic inflexibility (Galgani, Moro, et al., 2008).

Therefore, metabolic inflexibility in insulin-resistant patients is not explained by a primary impairment in glucose oxidation but instead the consequence of impaired glucose transport (Galgani, Moro, et al., 2008).

Insulin response to a meal

Glucose is the main driver to insulin secretion, however, it is also dependent on the relative proportions of macronutrients, the physical form in which the foods are ingested, and those factors influencing response to oral glucose alone (Wilcox, 2005).

Insulin response hinges on the glycaemic index of the food. When a food with a low glycaemic index is consumed, the rate of insulin secretion tends to be lower and digestion and the conversion to glucose requires more time (Wilcox, 2005). Although carbohydrate is the principal factor influencing glycaemic response to a meal, the addition of fat and protein to carbohydrate is positively associated with lowering glycaemic response (Moghaddam, Vogt, & Wolever, 2006). Therefore, in insulin-sensitive persons, the higher the fat and protein added to a carbohydrate meal, the lower will be the blood glucose concentration peak and consequently the insulin response (Ercan, Gannon, & Nuttall, 1994; Hollenbeck, Coulston, & Reaven, 1988; Moghaddam et al., 2006; Wilcox, 2005).

3.2. Free Fat Acids (FFA)

Free fatty acids are the vehicle by which triacylglycerol stored in adipose tissue is transported to its sites of utilization (Karpe, Dickmann, & Frayn, 2011). Plasma FFA plays an important role as the primary energy source for liver, pancreas, the resting state of skeletal muscle, and myocardium (Boden & Shulman, 2002). However, increased consumption of high-fat, energy-dense diets, particularly rich in saturated fatty acids, tend to be associated with insulin resistance and other metabolic diseases (Manco, Calvani, & Mingrone, 2004; Wilcox, 2005).

The excessive intake of lipids leads to a rise in plasma FFA concentration and an accumulation of triglycerides in many tissues, particularly in the adipose tissue (Manco et al., 2004). High plasma FFA concentration drives the enhanced lipid fuel oxidation, which impairs insulin-stimulated glucose disposal rate and decreases intracellular glucose (Galgani, Moro, et al., 2008). Additionally, fat cells do not have an unlimited capacity to expand and the over-expansion of existing fat cells creates inflammation. This, leads to insulin resistance within the fat cell, resulting in a net spillover of FFA to non-adipose tissues such as muscle, liver, and pancreas, which are unable to safely store large amounts of fat (Manco et al., 2004; Sears & Perry, 2015). Consequently, the development of lipotoxicity begins, which is the excess of lipid accumulation in the non-adipose tissue that leads to the real metabolic consequences of insulin resistance (Sears & Perry, 2015).

3.3. Mitochondrial oxidative capacity

Mitochondria is an organelle present in almost all eukaryotic cells that, besides having a central role in the execution of diverse cellular events, is responsible for cellular energy production (Boudina & Graham, 2014; McBride, Neuspiel, & Wasiak, 2006; Osellame, Blacker, & Duchen, 2012). Mitochondria, through ß-oxidation and the Krebs cycle, transforms

metabolic intermediates such as pyruvate, fatty acids, and amino acids into the reduced energetic equivalents NADH and/or $FADH_2$ (Smith et al., 2018).

Since it regulates glucose metabolism and fatty acid oxidation in most cell types, mitochondria fulfill a crucial role in determining cellular, tissue, and systemic MF (Smith et al., 2018). Nevertheless, despite the evidence indicating mitochondrial defects as a driving factor to insulin resistance and consequently metabolic inflexibility, the causal link between the two remains to be fully established (Galgani, Moro, et al., 2008; Karpe et al., 2011). Even though the reduction in mitochondrial oxidative capacity is linked to the development of obesity and type 2 diabetes mellitus, exercise, by increasing the number and density of mitochondria, is related to a better MF and insulin sensitivity (Muoio, 2014; Smith et al., 2018).

3.4. Skeletal muscle

Skeletal muscle represents the body's largest organ mass, however, at rest it has a low metabolic rate per mass unit compared with other organs, contributing for 40% to 50% of the variability in whole-body metabolic rate (Zurlo, Nemeth, Choksi, Sesodia, & Ravussin, 1994).

Skeletal muscle, oxidative or glycolytic, responds differently to fatty acids or glucose consumption. Oxidative fibers have more mitochondria and a higher concentration of oxidative enzymes, allowing to have a higher rate of fat oxidation compared with glycolytic fibers (Galgani, Moro, et al., 2008). Oxidative capacity of skeletal muscle may be essential to boost lipid oxidation to the level of lipid supply, plus if skeletal muscle cannot match lipid oxidation to fat uptake, it will lead to lipid accumulation, which in turn will cause insulin resistance as previously explained (Galgani, Moro, et al., 2008). The greater the percentage of oxidative fibers the higher will be the rate of fat oxidation, preventing insulin resistance. Consequently, insulin sensitivity can also be modulated by the oxidative capacity of skeletal muscle (Galgani, Moro, et al., 2008).

4. Aging

Aging is an inevitable and complex process characterized by the progressive degeneration of organ systems and tissues that is largely determined by genetics and influenced by a wide range of environmental factors (Nigam, Knight, Bhattacharya, & Bayer, 2012). A sedentary lifestyle increases as people age and is one of the underlying causes of age-related metabolic pathophysiology (Smith et al., 2018). Besides the more sedentary lifestyle, the aging process involves alterations in cardiovascular physiology and capacity, pulmonary function and respiratory capacity, neural function, endocrine functions, and body composition (McArdle et al., 2015). The alterations and deterioration of organ systems and tissues is the main risk factor for major age-related diseases including cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases (López-Otín, 2013). Alterations in body composition, as the increase of waist circumference, body fat percentage, and the development of sarcopenia – commonly used to describe the loss of skeletal muscle mass and strength that occurs in concert with biological aging, are also characteristics of aging (Doherty, 2003; López-Otín, 2013; McArdle et al., 2015). These body composition alterations coupled with endocrine changes cause deleterious effects on metabolism, such as the increase of fasting RQ, insulin resistance, the risk for developing diabetes, and decreased variance in daily RQ (Flack et al., 2010; López-Otín, 2013; McArdle et al., 2015). For these reasons, the elderly, MF tends to be compromised (Calçada et al., 2014).

Gaps in research and objective

Given MF associations with insulin sensitivity, many studies have been conducted around MF (Goodpaster & Sparks, 2017). These studies correlate the decrease in RQ variance and the increase in plasma insulin concentration variance with the interaction between lifestyle and biological factors.

The effects of distinct diets and weight loss have been reported to improve MF, however, although it is known that PA and physical exercise may have a positive impact on the general population, little attention has been given to the impact of the adoption of sedentary behaviors, physical inactivity, and detraining on MF (Bergouignan et al., 2013; Bergouignan et al., 2011; Rynders et al., 2017). Even knowing that these behaviors are increasingly present in today's population, especially in the older adults (65+) (Baptista et al., 2012; Judice et al., 2015; World Health Organization, 2014), the majority of studies are focusing on the adult population (up to age 64). Additionally, studies performed in older adults are predominantly cross-sectional, with limited understanding of the impact of sedentary behavior and reduction of PA on different biomarkers in older adults (Wirth et al., 2017). For this reason, it is relevant to do more experimental research in this population and examine the role of PA and sedentary behaviors on MF regulation (Smith et al., 2018).

Therefore, the purpose of this study was to evaluate the effects of a two-week interruption of habitual supervised and structured exercise sessions on MF, in trained older adults. The hypothesis was as follows: after the two weeks of detraining there would be a smaller variance in RQ and a higher variance in insulin after a 100 g oral glucose load, indicative of a lower MF.

Methodology

1. Recruitment process

Participants were recruited between November 2017 and March 2018 to take part in an intervention study for older adults. Media advertisements and attendance to local exercise classes were used to recruit the participants within the region of Oeiras –Portugal. Interested participants carried out the enrolment process (Fig. 2), starting with a recruitment session, where it was provided a thorough explanation of the intervention. In this presentation, participants had access to the following information: the main goals, details of the intervention in which they would engage and requirements to be a part of the study in terms of schedule and time availability. At the end of the presentation participants filled in a questionnaire to ascertain who met the inclusion criterion (appendix A). Written informed consents were obtained from all participants before and prior to any protocol-specific procedures (appendix B). This study was approved by the Ethics Committee of the Faculty of Human Kinetics and conducted in accordance with the Declaration of Helsinki an also with Convention on Human Rights and Biomedicine.

In order to be a part of this intervention, the participants had to be aged between 65 and 90 years, physically active, and engaged in structured exercise at least twice a week, for the past 6 months. People who had type 2 diabetes or any type of severe limitation that would prevent them from practicing exercise were excluded from the sample. Power and sample size calculations (G*Power 3.1.9.2) were based on an effect size of 0.78 for the glucose iAUC, while using the t-test for paired samples (two-tailed), a power of 0.80, and a significance of 0.05 (Hawari et al., 2016). The calculation yielded a sample size of 15 participants while expecting a dropout rate of 10%. A total of 12 participants were recruited and enrolled in the intervention (Fig. 2).

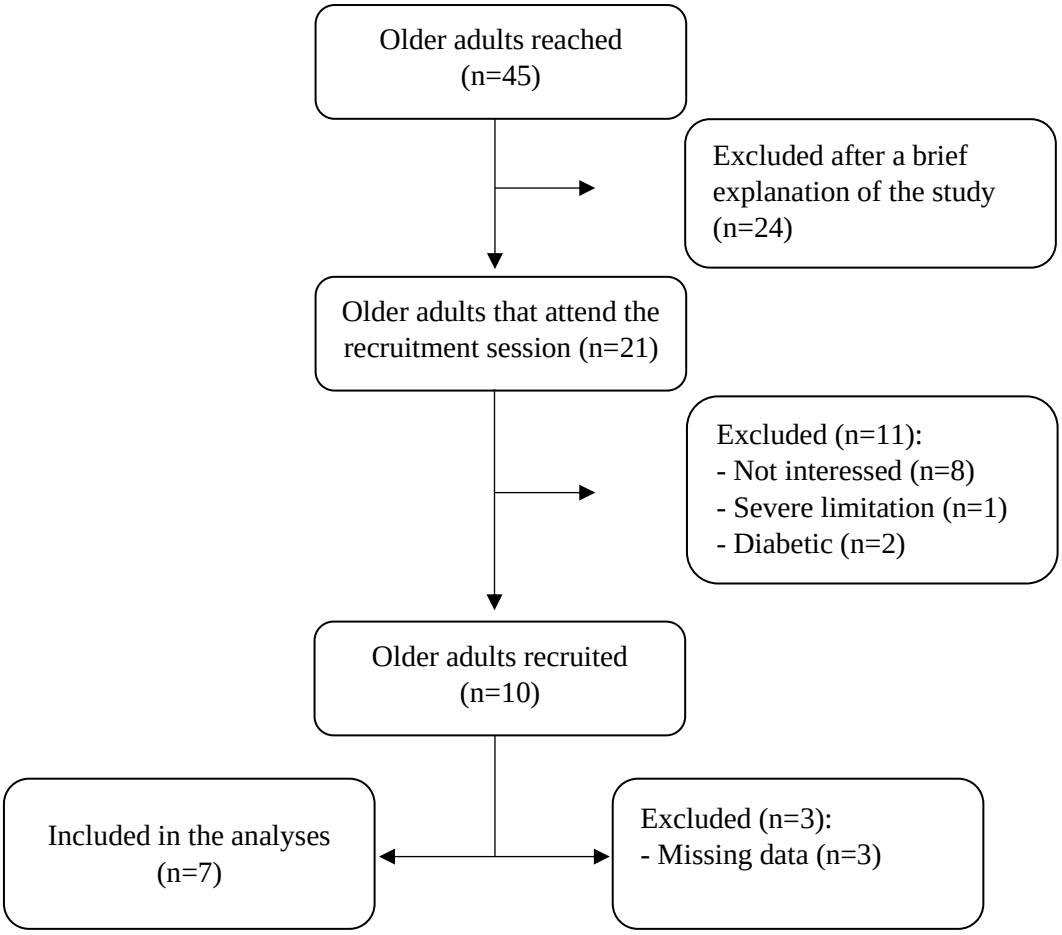

Fig. 2. Flowchart of participant recruitment.

2. Study design

Participants were followed in a crossover experimental design. MF, anthropometric measures, body composition measurements, and cardiorespiratory fitness were performed at baseline and after two weeks of detraining. Each evaluation moment (i.e. baseline and after two weeks of detraining) was completed on two different days. This intervention took place in *Faculdade de Motricidade Humana, Universidade de Lisboa*, and occurred over the course of 6 months.

Three master dissertations with distinct outcomes – one for glycaemic control, other about metabolic flexibility, and the last one regarding phase angle and muscle strength - were accomplished based on the data from this intervention. However, the primary outcome of the

intervention concerns changes in iAUC glucose. In the first day of assessments, each person received their schedule in the paper, which contained details about the location, time, and the activities to complete (***Fig. 3***).

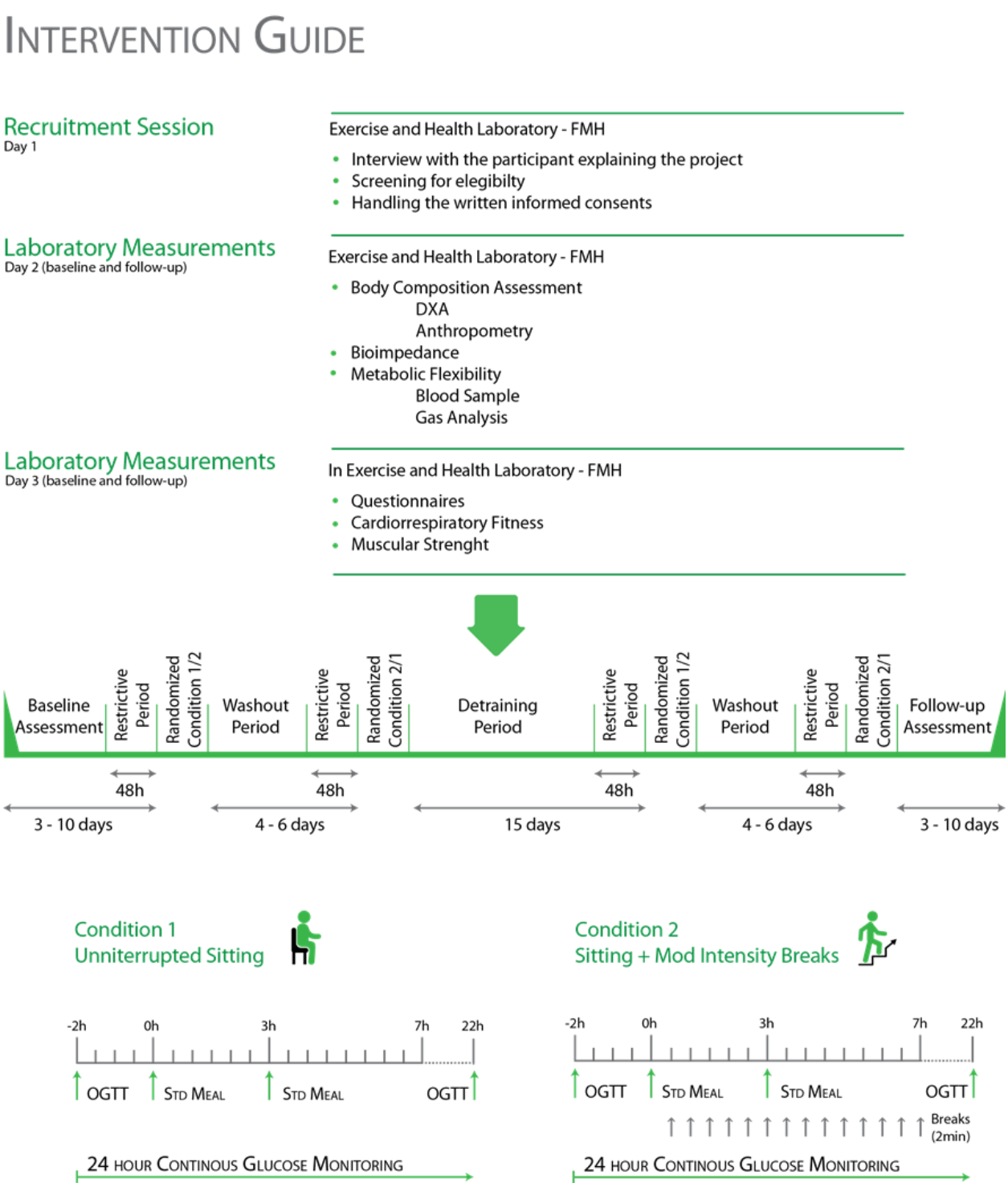

Fig. 3. Intervention guide comprising all the assessments and protocols from the three studies included in the intervention

3. Baseline and follow – up assessments

3.1. Anthropometric measures

3.1.1. Height and weight

Participants were weighed on an electronic scale without shoes wearing minimal clothing to the nearest 0.01 kg (Seca, Hamburg, Germany). Height was measured to the nearest 0.1 cm with a stadiometer (Seca, Hamburg, Germany), according to standardized procedures (Lohman, Roche, & Martorell, 1988).

3.2. Body composition measurements

Dual-energy X-ray absorptiometry (DXA) (Hologic Explorer-W, fan-beam densitometer, software QDR for Windows version 12.4, Waltham, USA) was used to estimate total FM (kg and %), abdominal FM (kg), FFM (kg), bone mineral content (BMC in kg) and BMI (kg/m^2). A whole-body scan was performed and the attenuation of X-rays pulsed between 70 and 140 kV synchronously with the line frequency for each pixel of the scanned image that was measured. Abdominal and gynoid body FM were measured through partial analyses of the DXA scan, based on regions of interest (ROIs) set by default on the DXA settings. Following the protocol for DXA described by the manufacturer, a phantom with six fields of acrylic and aluminium of varying thickness and known absorptive properties was scanned alongside each participant to serve as an external standard for the analyses of different tissue components. The same laboratory technician positioned the participants, performed the scans and executed the analyses according to the operator's manual using the standard analysis protocol. Based on ten participants, the CV in our laboratory for FM and abdominal FM were 1.7% and 0.01%, respectively.

3.3. Cardiorespiratory fitness

Cardiorespiratory fitness was determined using a modified Bruce protocol (Noonan & Dean, 2000) on a motorized treadmill to exhaustion (model Q-65, Quinton, Cardiac Science Corp; Bothell, WA, USA). Prior to the test, participants were familiarized with the protocol and with the Borg Rating of Perceived Exertion Scale (RPE) (Borg, 1982). At the end of each stage, they were requested to rate their perceived exertion using the Borgs scale. All graded exercise tests were monitored using a 12-lead electrocardiogram PC-based acquisition module by a certified cardiologist, and all data, including heart rate, were monitored and recorded using Omnia software. Inspired and expired gases were continuously analyzed, breath-by-breath, through a portable gas analyzer (QUARK RMR w/CPET, version 9.1, Cosmed, Rome, Italy). Participants exercised until at least two of the following test termination criteria were reached: (1) participants volitional fatigue; (2) respiratory exchange ratio reached 1.1 or higher; (3) participants reached predicted maximal heart rate; (4) oxygen uptake did not increase in spite of increasing workload (American College of Sports et al., 2018; Milani, Lavie, Mehra, & Ventura, 2006). The highest 20 seconds value for peak oxygen consumption (ml/kg/min) attained in the last minute was used in the analysis.

3.4. Metabolic flexibility

Metabolic flexibility was assessed with the ventilated hood method (QUARK RMR w/CPET, version 9.1, Cosmed, Rome, Italy) using indirect calorimetry to analyze variations in the respiratory quotient (RQ) (Wopereis et al., 2017). Participants arrived at the laboratory in the morning after an overnight fast of 10 h (Corpeleijn et al., 2008), where they were directed to the room of assessments with low lighting and with air temperature set between 20ºC-25ºC (Compher, Frankenfield, Keim, & Roth-Yousey, 2006). The head of the participant was covered by a transparent plastic canopy hood, which was connected to a blower that generates

a constant flow through the hood (Wopereis et al., 2017). During this process, participants kept inhaling air from the surrounding environment (room air), while exhaled O_2 and CO_2 content was measured by the equipment to provide a calculation for the O_2 consumption and CO_2 production (Wopereis et al., 2017). These specific values were used to calculated RQ by using the ratio of CO_2 exhaled to the amount of oxygen consumed by the individual ($RQ=VCO_2/VO_2$).

The test had a duration of 2 h and 30 min, considering that the first 30 min would correspond to the resting metabolic rate. After the first 30 min, there was a blood collection while the participant was still in a fasting condition. Right afterward the participants had to consume a solution within 5 min (Wopereis et al., 2017), composed of 100 g of glucose dissolved in 200 ml of water. In general, metabolic flexibility is measured through the change in RQ from the fasting to insulin-stimulated states, defined by the last minutes of the clamp (Malin et al., 2013; Stull, Galgani, Johnson, & Cefalu, 2010), or according to insulin and RQ responses to a standard meal (Baig et al., 2016; Bergouignan et al., 2013). However, both insulin and RQ have faster and more marked response when glucose is singly consumed (Gómez, Jéquier, Chabot, Büber, & Felber, 1972; Meier et al., 2009), being even more noticeable when the solution is composed of 100 g of glucose instead of 75 g (Castro, Scott, Grettie, Macfarlane, & Bailey, 1970; Soonthornpun, Soonthornpun, Aksonteing, & Thamprasit, 2003).

Since 100 g oral glucose load had a faster response, RQ and insulin can reach its peak within 45 to 120 min after drinking the solution (Bloesch, Schutz, Breitenstein, Jéquier, & Felber, 1988; Bratusch-Marrain, Waldhäusl, Gasić, Korn, & Nowotny, 1980; Charriere, Montani, & Dulloo, 2016; Dirks et al., 2018; Kahn et al., 1990; Meier et al., 2009; Simonsen, Bulow, Madsen, Hermansen, & Astrup, 1993). After ingesting the glucose solution RQ and substrate oxidation were continuously measured throughout the 2 h exam, with blood collection

every 60 min (i.e. fasting, 60 min and 120 min) (Al-Jaouni, Schneider, Rampal, & Hebuterne, 2002; Wopereis et al., 2017). Participants recorded their food intake in the day prior to the baseline test and were instructed to eat the same meal on the follow-up exam.

In order to smooth the curve for RQ, since the data provided by QUARK RMR is given with a frequency of 10 seconds, five points with half-hour intervals were defined -1, 30, 60, 90 and 120 min (Kardinaal et al., 2015). Fasting RQ value, represented by -1, was obtained during the 30 min of resting metabolic rate measurement, in which the first 15 min of adaptation period under the hood were discarded (Stull et al., 2010). From the subsequent 15 min, the average of the lowest 5 consecutive min values with a coefficient of variation equal to or less than 5% was considered (Feurer & Mullen, 1986). The remaining points 30, 60, 90 and 120 min were represented by the average of previous 5 min periods measurement (Fernandez-Verdejo, Bajpeyi, Ravussin, & Galgani, 2018).

4. Detraining period

After completing all the baseline assessments, participants underwent a detraining period of two weeks. During this period, participants were instructed to refrain from structured and supervised exercise sessions (Esain, Gil, Bidaurrazaga-Letona, & Rodriguez-Larrad, 2018) at their local gym classes, and were also advised to reduce their daily levels of physical activity (e.g. avoid long walks). All physical activity performed by the participants during these two-weeks was monitored by an accelerometer.

At the end of the detraining period, participants underwent through follow-up assessments which consisted of the same assessments that they performed at baseline.

5. Objective measures of sedentary time and PA

Sedentary time and PA were assessed by accelerometry (ActiGraph, GT3X model, Fort Walton Beach, FL) in two moments: during participants free living before the detraining period and during detraining period (2-weeks). The accelerometer is a small device that measures the acceleration of normal human movements, ignoring high-frequency vibrations associated with mechanical equipment. All participants were asked to wear the accelerometer on the right hip, close to the iliac crest. The devices were activated on the first day (in the morning) and data were recorded in 60 seconds epochs. Apart from accelerometer non-wear time (i.e., when it was removed during sleep and bathing activities), periods of at least 60 consecutive minutes of zero activity intensity counts were also considered as non-wear time (Colley, Connor Gorber, & Tremblay, 2010).

A valid day was defined as 600 min (10 h) or more of monitored wear time, and all participants were instructed to wear the equipment during the detraining period (2-weeks). If the participants were unable to use it throughout the detraining period, they had to use the equipment at least 3 valid days (including one weekend day). The device activation, download, and processing were performed using the software Actilife (v.6.9.1). The cut-off values used to define the intensity of PA and therefore to quantify the meantime in each intensity (sedentary, light, moderate or vigorous) were as follows: sedentary: < 100 counts·min-1; light: 100-2019 counts·min-1; moderate: 2020-5998 counts·min-1 (corresponding to 3-5.9 METs); vigorous: ≥ 5999 counts·min-1 (corresponding to ≥ 6 METs) (Troiano et al., 2008). There are no cut-offs for the sedentary-time using the three-axial information from this new generation Actigraph GT3X+ accelerometer; therefore, we used the previous cut-offs based on the vertical axis only.

6. Laboratory measurements

A certified health care professional performed all the blood sample collections. The participants had their blood collected in a seated position from the antecubital vein, into dry tubes and into tubes containing ethylenediaminetetraacetic acid (i.e. anticoagulant).

Biological samples were centrifuged, after 20 min of collection, at 3000 g and at 4ºC for 15 min. Aliquots were frozen in Faculdade de Motricidade Humana in order to use in the future strictly for the purpose of research. After that, the blood samples were stowed and processed in the *Associação Protetora dos Diabéticos de Portugal* (APDP) and were properly labeled with codes for each participant and moment of collection (to assure the data confidentiality).

7. Calculations and statistical analyses

Statistical analyses were performed using IBM SPSS version 25.0 for Windows. Means and standard deviations were calculated for body composition, cardiorespiratory capacity, blood variables, and for all the RQ points. In order to verify if there were differences between baseline and post-detraining moments in these variables, t-tests for paired data were used. A general linear model for repeated measures was used to adjust the changes in each RQ and insulin points before and after detraining, for the covariates sex and age. The Greenhouse-Geisser correction was used to analyze the results. Regarding how MF was affected by detraining, RQ and insulin variance were determined, for each participant, according to the method used by Bergouignan et al. (2013). Then, to compare the differences between moments, a general linear model for repeated measures was used adjusting for the covariates sex and age, results were analyzed according to the Greenhouse-Geisser correction. A P-value of less than 0.05 was considered as statistically significant.

Results

1. Participants Characteristics

Of the 12 participants who fulfilled all the evaluations and protocols, five were excluded, two for being diabetic and three for not having all the necessary data, which did not allow comparisons between the two moments.

In the table below it is shown, according to the data obtained by the accelerometer before and during the 2-weeks detraining, the mean PA levels recorded by all the participants. The PA results are present in min/day of sedentary time (ST), low-intensity PA (LIPA), MVPA, and steps/day. There was a complication with the accelerometer data from one of the participants in which the data during the detraining period was lost.

It is possible to note that the participants, in mean, had overall 176 steps/day less at baseline than during the detraining period, comprising of 17 min/day more sedentary time, and 23 min/day less of low-intensity PA. Moderate and vigorous PA did not suffer any difference in the mean values. Individually, 2 participants fulfilled the 30 min of MVPA and were considered physically active according to the ACSM recommendations during the detraining period, and 3 of the 7 individuals met these criteria on their daily basis. Thus, with the negligible differences in PA between the two moments, we can perceive that, during detraining, the participants did not reduce their daily levels of physical activity as advised, neither compensated their exercise sessions with more MVPA.

Table 1. PA data measured on a daily basis, before and during the 2-weeks of detraining

	Daily Basis	Detraining Period	Dif
ST (min/day)	582 ± 38	565 ± 91	17
LIPA (min/day)	239 ± 60	262 ± 78	23
MVPA (min/day)	27 ± 22	27 ± 28	0
Steps/day	6273 ± 2404	6449 ± 3160	176

ST, sedentary time; LIPA, low-intensity physical activity; MVPA, moderate and vigorous physical activity; Dif, difference between the two moments

Participants characteristics at baseline and post-detraining are presented in Table 2. The 7 older adults included in the study were 76.9 ± 6.5 years old, height about 1.62 ± 0.06 cm and weight 70.6 ± 7 kg.

Even though there were no differences between moments for any of the variables (P-value ≥ 0.05), there are perceptive changes in some of them. Resting metabolic rate (RMR) decreased 6.3%, which represents less 108.3 kcal spent per day. Cardiorespiratory capacity suffered a decrease of 13.5%, decreasing VO_{2max} about 3.1 ml/kg/min. Body composition (weight, BMI, fat mass, free fat mass) and blood biomarkers (HbA1c, fasting glucose, fasting insulin, fasting total cholesterol, fasting HDL, fasting LDL, fasting triglycerides) did not change with the short-term detraining.

Table 2. Baseline and post-detraining characteristics of study participants

	Baseline	Post-detraining	Dif
Age (years)	76.9 ± 6.5		
Weight (kg)	70.6 ± 7	70.8 ± 7.2	0.2
BMI (kg/m²)	26.7 ± 2.2	26.8 ± 2.27	0.1
Fat Mass (kg)	23.59 ± 5.1	23.75 ± 4.9	0.16
Trunk Fat Mass (kg)	13.02 ± 2.14	13.44 ± 1.87	0.42
Free Fat Mass (kg)	45.8 ± 6.3	46.2 ± 6.8	0.4
VO_{2max} (ml/kg/min)	25.1 ± 1.97	21.7 ± 4.61	-3.4
RMR (kcal/day)	1718.8 ± 216.7	1610.51 ± 218.9	-108.3
HbA1c (%)	5.56 ± 0.25	5.63 ± 0.34	0.07
Fasting			
Glucose (mg/dL)	96.96 ± 7.9	97.04 ± 9.54	0.08
Insulin (µUI/mL)	9.94 ± 4.24	8.34 ± 4.41	-1.6
Total Cholesterol (mg/dL)	185.6 ± 28	170.4 ± 42.82	-15.2
LDL-Cholesterol (mg/dL)	122.5 ± 26.78	113 ± 35.12	-9.5
HDL-Cholesterol (mg/dL)	55.98 ± 6.4	52.52 ± 7.8	-3.46
Triglyceride (mg/dL)	85.8 ± 14.72	81.47 ± 16.5	-4.33

BMI, body mass index; Dif, the difference between the two moments; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RMR, resting metabolic rate; VO_{2max}, maximal oxygen uptake

*Significantly different from baseline

2. Metabolic Flexibility

In Table 3, baseline and post detraining values for MF variables – RQ and insulin, and the difference between the two moments for these variables are shown.

Table 3. Metabolic Flexibility - RQ and Insulin changes

	Baseline	Post-detraining	Dif
Respiratory Quotient			
Fasting RQ	0.71 ± 0.04	0.74 ± 0.08	0.03[a]
30 min RQ	0.69 ± 0.02	0.73 ± 0.08	0.04
60 min RQ	0.76 ± 0.05	0.77 ± 0.09	0.01
90 min RQ	0.75 ± 0.09	0.77 ± 0.07	0.02
120 min RQ	0.74 ± 0.09	0.79 ± 0.07	0.05
Insulin (µUI/mL)			
Fasting Insulin	7.08 ± 4.37	6.28 ± 3.67	-0.8
60 min Insulin	67.92 ± 47.84	70.08 ± 23.69	2.16
120 min Insulin	69.23 ± 32.73	75.85 ± 28.12	6.62

[a] Significantly different from baseline adjusted for sex and age (P-value < 0.05);

2.1. Description for the response to the oral glucose load on each moment

Baseline

At baseline, RQ reached its peak 60 min after the oral glucose load, with a value of 0.76 about 0.07 higher than the previous 30 min, and 0.05 higher than the fasting RQ. In the following hour, the RQ suffered a reduction of 0.01 every 30 minutes.

Regarding baseline insulin, it suffered an increase of 60.84 µUI/mL in the first hour (7.08 µUI/mL in fasting to 67.92 µUI/mL in the first hour) and although it only reached its peak at 120 min with 69.23 µUI/mL, this value was only 1.31 µUI/mL higher than the previous hour.

Post-detraining

In post-detraining RQ progressively increases, only reaching its peak value (0.79) 120 minutes after the drink, with a rise of 0.05 over fasting value. Post-detraining insulin increased 63.8 µUI/mL from fasting (6.28 µUI/mL) to the first hour (70.08 µUI/mL). The highest value (75.85 µUI/mL) was achieved after two hours of the oral glucose drink and was about 5.77 µUI/mL greater than the previous hour.

2.2. Differences for the responses to glucose load between the two moments

Fig. 4 presents the responses of both insulin and RQ to 100 g oral glucose load in both moments. When adjusted for sex and age, there were only significant differences between the two moments in fasting RQ (P-value=0.044). Nevertheless, there was a trend for all the RQ and insulin mean values to be higher after the detraining period, with the exception of fasting insulin. The 30 and 120 min RQ are about 0.04 and 0.05 increased in post-detraining, showing a rise of 5.8% and 6.8%, respectively.

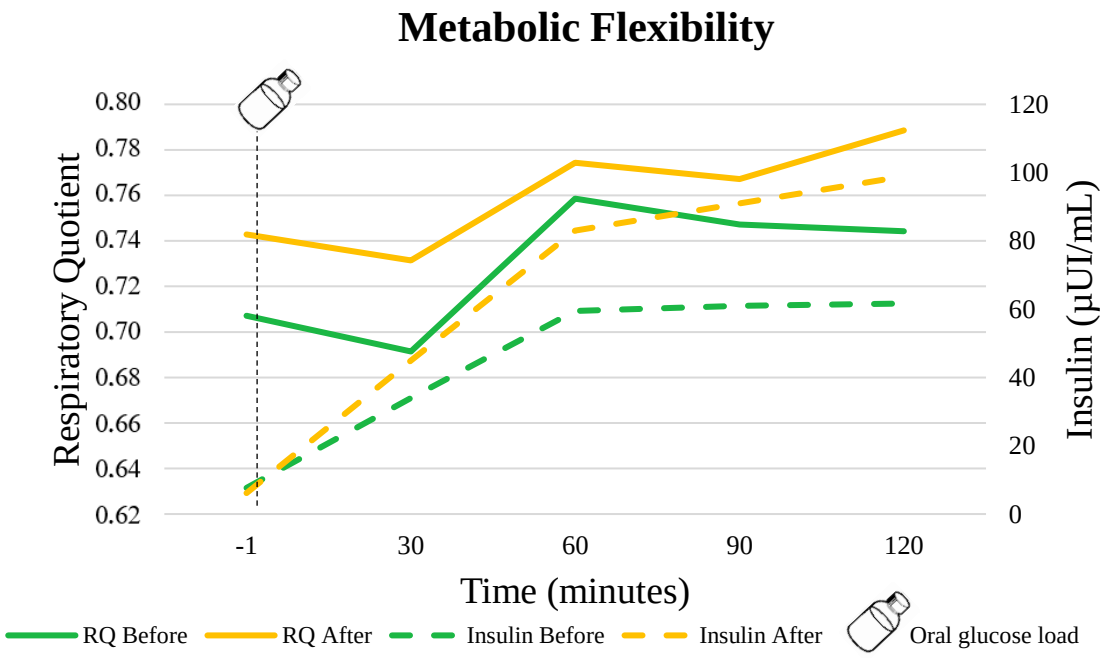

Fig. 4. Time course of NPRQ and plasma insulin concentrations before and after detraining.

Despite post-detraining having a slower response to the oral glucose load, only reaching its highest RQ value 60 min after the baseline peak, both moments had a 0.05 difference between peak and fasting value RQ.

To further address how MF was affected by detraining, the relationship between variance in insulin and RQ was determined in both moments, as illustrated in Fig. 5.

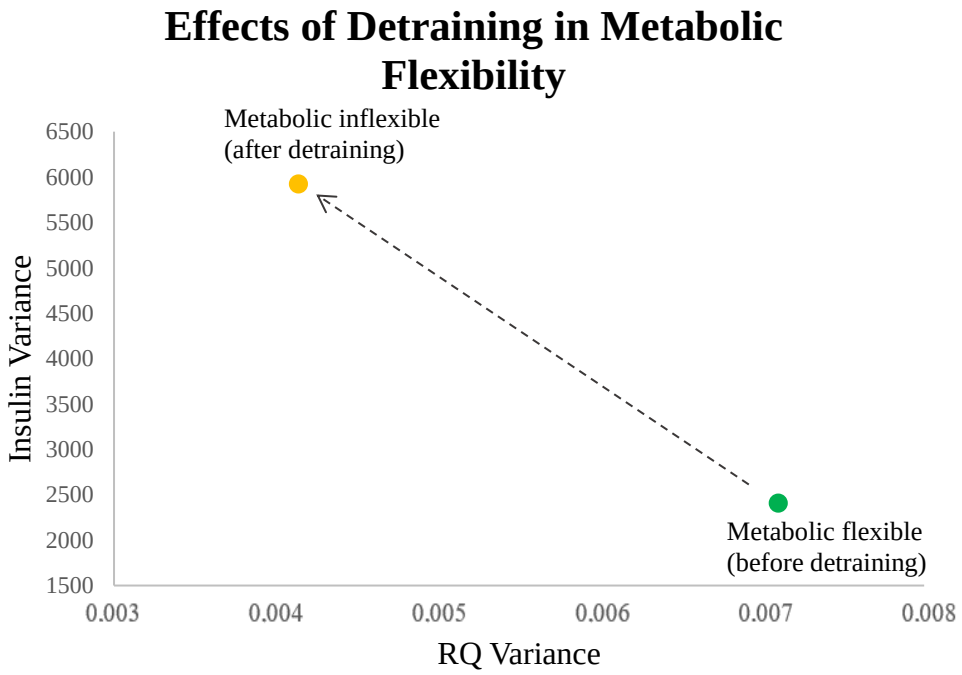

Fig. 5. Relationship between variance in insulin and variance in RQ during the training stage - before detraining period and after the short-term detraining.

Fig. 5 shows that at baseline participants had a higher RQ variance (0.0071) and a lower variance in insulin (2405). In contrast, after detraining the same participants had a decrease of 41.7% in RQ variance and an almost threefold increase in insulin variance. Despite these changes, there were no significant differences between the two moments (P-value for RQ variance = 0.231; P-value for Insulin variance = 0.692).

Discussion

In the present study, we assessed the isolated impact of 2 weeks of detraining without any changes in diet (through the maintenance of their usual and balanced diet and the record of their food intake in the previous day of the baseline test and its replication in the day before the post detraining MF assessment) in seven older trained adults (i.e. three woman and four man).

To our knowledge, this is the first study that investigated the effects of a two-week interruption of habitual supervised and structured exercise sessions on MF in previously trained older adults. Effectively, MF studies were only conducted in adults and not using the response to a 100 g oral glucose load. For this reason, it is difficult to compare the results to other scientific studies. Consequently, the discussion will focus on similar studies that may have a different populations and a different method to evaluate RQ and insulin responses.

1. Baseline metabolic flexibility

Concomitant with other studies, it was observed a decrease between fasting and 30 min RQ (Charriere et al., 2016; Simonsen et al., 1993). This fall in RQ is unlikely to be caused by a shift to lipid oxidation since the glucose consumption exerts an antilipolytic action (Simonsen et al., 1993). The delayed switch from fat to carbohydrate oxidation may be explained by the inability to consume the 100 g of glucose during the measurement of the gas exchange, being the participant thereby required to leave the transparent plastic canopy hood. For this reason, when the ventilated hood was replaced, the CO_2 in the blower that generates a constant flow through the hood may have decreased, explaining the negligible decrease in 30 min RQ.

Regardless of the slight decrease in 30 minutes RQ, the RQ peak was reached 60 min after the consumption of the oral glucose load (Bloesch, Schutz, Breitenstein, Jequier, & Felber, 1988; Charriere et al., 2016), with a value of 0.76. The RQ may have reached the peak

60 min post-drink due the fact that both insulin and RQ have faster and marked responses when glucose is consumed isolated (Gómez et al., 1972; Meier et al., 2009), even though in general, MF is measured through the change in RQ from the fasting to insulin-stimulated states, defined by the last minutes of the clamp (Malin et al., 2013; Stull et al., 2010), or according to insulin and RQ response to a standardised meal (Baig et al., 2016; Bergouignan et al., 2013). The insulin and RQ response is even more noticeable when the solution is composed per 100 g of glucose instead of 75 g (Castro et al., 1970; Soonthornpun et al., 2003), which was the case in our study.

In this study it was observed a 0.05 difference between the fasting RQ and the peak value, similar to the response to 60 g of glucose in twelve healthy young non-obese adults reported by Charriere et al. (2016), and to the response to a high carbohydrate meal (400 ml liquid meal with 600 kcal composed by 56% of total energy from carbohydrate), in eighteen young adults (Baig et al., 2016; Charriere et al., 2016). This may occur because it is expected that older adults have a lower RQ (Bloesch, Schutz, Breitenstein, Jequier, et al., 1988) and a higher insulinemic response (Bloesch, Schutz, Breitenstein, Jequier, et al., 1988) than younger adults. Despite alterations in metabolic response to fuel availability with age are not well understood, age, body composition changes (BMI, FM, FFM, waist circumference), hormonal changes, insulin sensitivity, dietary composition, and energy balance have been pointed out as determinant factors of the changes in substrate oxidation and metabolic flexibility (St-Onge & Gallagher, 2010; Weyer, Snitker, Rising, Bogardus, & Ravussin, 1999). For this reason, the response of the elderly to 100 g of glucose can equal that of 60 g of glucose or even a mixed meal in adults.

According to Perley & Kipnis (1967), Meier et al. (2009) and Matsuda & DeFronzo (1999), in adults it was expected that insulin reaches its peak 60 min after glucose load (Matsuda & DeFronzo, 1999; Meier et al., 2009; Perley & Kipnis, 1967). However, Kahn et

al. (1990) reported that in older adults insulin response was slower, only reaching the peak value 120 min after the 100 g oral glucose load (Kahn et al., 1990). In the present study, insulin also merely reached its highest value at 120 min after the 100 g oral glucose load. Notwithstanding, this value was not significantly higher than the one in the first hour.

2. Post-detraining metabolic flexibility

Given that, at baseline, the highest value was achieved after one hour and that both insulin and RQ have faster and marked responses when glucose is consumed isolated, post-detraining RQ, contrary to expectations, only reached the peak value after 120 min. It is worth mentioning that as the procedure of measuring MF through indirect calorimetry ended after 2 h, we could not ensure that the peak was effectively reached at 120 min or after that. Nevertheless, this delay to reach the RQ peak after the detraining period demonstrated less responsiveness to glucose. These results are similar to other studies that use different approaches to measure MF (Bergouignan et al., 2013; Rudwill et al., 2018). Rudwill et al. (2018) reported a progressive increase in RQ that also achieved the highest value 2 h post-drink. However, it is important to underline that their participants were adults and they use a moderately high-fat, high-energy test meal composed by 44% carbohydrate, 41% lipid, 15% protein which is expected to have a slower metabolic response compared to glucose (Rudwill et al., 2018).

Notwithstanding, the delay in the response to glucose, resemble what happened at the baseline, which was a 0.05 difference between fasting and the highest RQ value.

Concomitant with Kahn et al. (1990) and baseline results, in post-detraining our older adults participants merely reached the insulin peak 120 min after the 100g oral glucose load, which showed a slower insulin response compared to adults in Perley & Kipnis (1967), Meier

et al. (2009) and Matsuda & DeFronzo (1999) investigations (Kahn et al., 1990; Matsuda & DeFronzo, 1999; Meier et al., 2009; Perley & Kipnis, 1967).

3. Metabolic flexibility difference between the two moments

Adjusting for sex and age there were only significant differences between the two moments in fasting RQ, which demonstrated that regardless of sex and age the suspension of habitual and structured exercise sessions for two weeks causes an increase in fasting RQ.

Although there were only differences between the two moments in fasting RQ, a trend for all the RQ and insulin mean values being higher after the detraining period was noticeable. Therefore, the detraining period induced an overall increase in RQ, suggesting a greater utilization of carbohydrates as energy after detraining principally in the fasting condition. This finding is consistent with those from previous detraining and bed-rest studies, which reported that a reduction in PA, regardless of energy balance conditions, induces an increased reliance on carbohydrate as an energy substrate with a decrease in fat oxidation (Bergouignan et al., 2013; Blanc et al., 2000; Stein & Wade, 2005).

Additionally, as mentioned before, a lower RQ response in older adults was expected compared to adults, even though, at baseline, the older adults in our study were able to reach their highest RQ value 1 h after the 100 g of glucose. After the short-term detraining, a delay in the RQ response compared to the baseline was clearly noticeable, which demonstrates less responsiveness to the 100 g glucose load as a consequence of the detraining. These results resemble those of Rudwill et al. (2018), who demonstrated that 21 days of bed rest induced a decreased responsiveness to a mixed meal compared to baseline (Rudwill et al., 2018).

Furthermore, a metabolically flexible state is defined by a high capacity to switch fuel in response to feeding, shifting from fat to carbohydrate oxidation in association with small changes in insulin concentration, whereas a metabolically inflexible state is characterized as a

high variance in plasma insulin concentration and a low variance in RQ (Bergouignan et al., 2013; Rynders et al., 2017). Therefore, the difference between RQ and insulin variances before and after the two weeks of detraining was determined in order to compare the metabolic status of participants when they were trained *vs* after the two-weeks detraining. It was observed that after detraining, RQ variance in response to the oral glucose load decreased about 41.7% and insulin variance suffered an almost threefold increase. Although not statistically significant, both of these changes suggest a decreased MF with the suspension of habitual supervised and structured exercise sessions. This decrease in MF goes in accordance to the results reported by Bergouignan et al. (2013) and Rudwill et al. (2018) that also showed that a reduction in PA decreased RQ variance and increased insulin variance (Bergouignan et al., 2013; Rudwill et al., 2018).

As will be discussed below the explanatory mechanisms for this decrease in MF after short-term detraining are ambiguous. PA is one of the main determinants of energy expenditure, while inflow depends on the quantity and quality of meals, outflow can only be modulated by PA (Rynders et al., 2017). PA also favors the adjustment to fat oxidation with fat intake when dietary fat increases and is therefore fundamental to energy balance, weight control and obesity prevention (Rynders et al., 2017; World Health Organization, 2014). Thereby, it becomes difficult to discriminate whether these changes in MF may be attributable to physical inactivity *per se* or to the associated changes in energy balance.

Physical inactivity has been reported to lead to rapid loss of skeletal muscle mass especially in older adults (Esain et al., 2018; Smith et al., 2018; Wall & van Loon, 2013), reduce fasting and post-prandial lipid oxidation in favour of greater use of carbohydrate as fuel and ectopic fat storage (Bergouignan et al., 2013; Blanc et al., 2000), and is associated with an increase in plasma insulin concentration and the development of insulin resistance. All of these

changes suggest that physical inactivity alone is one of the main causes in the development of metabolic inflexibility (Bergouignan et al., 2013).

Biensø et al. (2012), reported that 7 days of bed rest leads to a 22% decline in insulin sensitivity, which was attributable to a direct effect on intracellular insulin signaling pathways (Biensø et al., 2012). Thus, the mechanisms behind physical inactivity–induced insulin resistance in skeletal muscle can be explained by the decreased actions of several fundamental proteins in insulin-signaling pathway (Bergouignan et al., 2011; Biensø et al., 2012; Dirks et al., 2018), such as, reduced muscle GLUT4 (insulin-regulated glucose transporter), hexokinase (the protein that phosphorylates glucose after its entrance in the cell), protein kinase B/Akt1 and Akt2 (responsible for the insulin-induced translocation of GLUT4 to the plasma membrane) (Bergouignan et al., 2011; Biensø et al., 2012). A recent study investigated the effect of 1 day of sitting with a level of physical inactivity, closer to that observed in strict bed rest, on insulin action (Stephens, Granados, Zderic, Hamilton, & Braun, 2011). They showed that with and without energy balance, reduced PA considerably alters insulin sensitivity. The fact that this effect still occurred when food intake matched energy expenditure emphasized the deleterious effects of physical inactivity alone (Bergouignan et al., 2011). Bergouignan et al. (2013) showed that both a reduction in spontaneous and structured PA and physical inactivity decreased the variance in daily RQ and increased that of insulin, both features of a decreased metabolic flexibility. They also suggest that physical inactivity per se is one of the primary causes in the development of metabolic inflexibility (Bergouignan et al., 2013).

In this study, even though the diet of the participants was not tightly controlled in terms of energy consumption and macronutrient balance, weight, BMI, and fat mass did not vary significantly, suggesting that participants reached a stable energy balance during the detraining period. These results suggest that the participants adjusted their energy intake to total energy expenditure to maintain energy balance during the short-term detraining.

Since the impact of the reduction of PA is increased in the aging population and taking into account the integrity of the participants, this study did not expose participants to extreme decreases in PA, which may have limited the significant findings in our results. Regardless, the development of metabolic inflexibility with detraining may be explained through the same mechanisms as that of bed rest and physical inactivity. Consequently, in conformity with Bergouignan et al. (2011), Rudwill et al. (2018) and Dirks et al. (2018), our study showed that physical inactivity *per se* is one of the primary causes involved in the development of metabolic inflexibility, even when changes in energy balance are not detectable (Bergouignan et al., 2011; Dirks et al., 2018; Rudwill et al., 2018).

Limitations

Despite the encouraging findings of this study, some limitations must be acknowledged.

Our greatest limitation was our small sample size, which limited our power to observe small to medium-sized effects. We hypothesised that if the sample size was larger, we would find significant changes, as we found pronounced trends.

Regarding the evaluation of MF, obtainment of five blood samples would have been ideal so that, as RQ, the variance of insulin could be evaluated with greater precision. However, due to the tremendous difficulty in obtaining three blood collections, we were unable to collect the desirable 5 samples. Moreover, after detraining, the 2 h of indirect calorimetry may not have been sufficient to reach the RQ peak, so future studies must extend this period of assessment.

It is also important to mention the physical activity assessment limitations. Two of the seven participants perform structured PA sessions in an aquatic environment, and the accelerometer does not record these data. The PA session plus the time spent bathing and dressing result in an accelerometer non-wear time of 60 min. Since the non-wear time was defined as 60 consecutive min of zero activity, the time spent during the structured sessions classes was considered as non-activity time. Furthermore, the remaining five participants perform strength exercises during their supervised class performed on the machines and in a sitting position that may have been measured as sedentary time (Sardinha, Magalhaes, Santos, & Judice, 2017). Therefore, participants PA during their daily basis may be underestimated and the decrease in PA during the detraining period might be higher than what was observed.

During detraining, despite the maintenance of their usual and balanced diet and the record of their food intake in the previous day of the baseline test and its replication in the day before the post detraining MF assessment, the diet of the participants was not tightly controlled during the full study period.

Conclusion

In conclusion, the suspension of habitual supervised and structured exercise sessions, for two weeks, was not sufficient to decrease MF among trained older adults. However, there is a trend to: (1) increase the reliance on carbohydrate as an energy substrate, which is associated with a decrease in fat oxidation, (2) slow the response to the 100 g oral glucose load, (3) reduce RQ variance and increase insulin variance in response to the oral glucose load. All of these trends indicate a reduced capacity to switch fuel in response to feeding, which is typically present in metabolically inflexible individuals.

Future work

Studies that tightly control the energy balance and macronutrient distribution in order to assess the isolated impact of decreased PA on MF in the elderly population are needed to confirm these results. Future studies are also needed to define cut-off values for RQ and insulin variance that define an individual as metabolically inflexible according to their age. It is also important to determine the PA threshold below which metabolic inflexibility is developed, so that a dose-response can be recommended.

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Appendix A - exclusion criteria questionnaire

QUESTIONÁRIO DE AVALIAÇÃO

Nome: _____

Género:. Feminino □ Masculino □ Data: ____/_____/ _____ Data de nascimento: _____/____/_____

Telefone: _____ Peso (kg): _____ Altura (m): _____

Fuma? Sim □ Quantos cigarros por dia? _____

Não □

DOENÇAS CRÓNICAS

Apresenta alguma das seguintes doenças?

Doenças cardiovasculares:

- □ Hipertensão arterial
- □ Enfarte do miocárdio
- □ Angina do peito
- □ AVC
- □ Colesterol elevado
- □ Osteoporose

Outra(s): _____

Outra(s): _____

Doenças Renais □ Doenças Pulmonares (Ex: Asma) □

Qual/Quais: _____

Doenças metabólicas:

- □ Artrite/Artrose
- □ Cancro
- □ Anemia
- □ Diabetes

Outra(s): _____

Teve alguma lesão nos últimos 6 meses? Sim □ Não □

Se sim, que lesão? _____

Ocorrência de Fraturas

N.º total de fraturas que sofreu ao longo da vida: _____ Local ósseo das fraturas: _____ _____

Ocorrência de quedas (últimos 12 meses)

Quantas vezes caiu no último ano? _____

Ocorrência das quedas:

☐ A realizar uma tarefa usual

☐ A realizar uma tarefa excepcionais ou de grande dificuldade
☐ A realizar uma actividade física supervisionada por um professor

Medicação

Alguma vez tomou algum medicamento mais de 6 meses? Que outros medicamentos toma atualmente? (* indicar para que doença o medicamento foi prescrito)

*	Medicamento	Ano de início	N.º de anos

Observações:

Saúde e Incapacidade Física

Apresenta algum dos seguintes problemas de saúde?

	Sim	Não
1. Incontinência urinária (perda de urina)	☐	☐
2. Tonturas frequentes	☐	☐
3. Problemas nos pés (inflamações, calos, etc)	☐	☐
4. Problemas de visão (não reconhece uma pessoa a quatro metros de distância mesmo usando óculos ou lentes de contacto).	☐	☐
5. Problemas da audição (não consegue seguir uma conversa de um grupo de quatro pessoas mesmo com aparelho auditivo)	☐	☐
6. Problemas de equilíbrio (algumas vezes tem dificuldade em equilibrar-se)	☐	☐

Quantas vezes foi ao médico no último mês? _____

Quantas vezes foi ou permaneceu no hospital nos últimos 6 meses? _____

Considera que a sua saúde é: Muito má ☐ Má ☐ Razoável ☐ Boa ☐ Excelente ☐

Notou perda de peso involuntária nos últimos 12 meses? ☐ Não ☐ Sim (peso inicial e peso perdido)

Tem tido falta de apetite? ☐ Não ☐ Sim

No último mês sentiu que tinha muito pouca energia para as coisas que pretendia realizar? ☐ Não ☐ Sim

Ocupações do tempo livre

Quais das seguintes atividades gosta de praticar no seu tempo livre?

☐ Ver televisão? ☐ Jogos de tabuleiro

- ☐ Jogar computador
- ☐ Ler um livro/jornais/revistas
- ☐ Ouvir música
- ☐ Jogar às cartas
- ☐ Fazer sudoku

Outras:

_____

Calendarização da Intervenção

Relativamente à Calendarização da Intervenção (pode assinalar mais do que 1 opção):

- ☐ Não posso realizar a intervenção se esta for durante o mês de Janeiro
- ☐ Não posso realizar a intervenção se esta for durante o mês de Fevereiro
- ☐ Não posso realizar a intervenção se esta for durante o mês de Março
- ☐ Não posso realizar a intervenção se esta for durante o mês de Abril
- ☐ Não posso realizar a intervenção se esta for durante o mês de Maio
- ☐ Não tenho preferência ou restrições relativamente a nenhuma das datas

Já participou em outro(s) estudo(s)?

- ☐ Sim. Se sim, qual?_____
- ☐ Não

Obrigado pela sua colaboração!

Appendix B - informed consent

CONSENTIMENTO INFORMADO LIVRE E ESCLARECIDO

Título do projeto: Efeitos da Interrupção do Comportamento Sedentário e da Inatividade Física na Resposta Pós-prandial da Glicémia, na Sensibilidade à Insulina, na Flexibilidade Metabólica e no ângulo de fase

Pessoa responsável pelo projeto: Professor Doutor Luís Bettencourt Sardinha

Instituição de acolhimento: Faculdade de Motricidade Humana – Universidade de Lisboa

Este documento, designado **Consentimento, Informado, Livre e Esclarecido**, contém informação importante em relação ao estudo para o qual foi abordado/a, bem como o que esperar se decidir participar no mesmo. Leia atentamente toda a informação aqui contida. Deve sentir-se inteiramente livre para colocar qualquer questão, assim como para discutir com terceiros (amigos, familiares) a decisão da sua participação neste estudo.

Este estudo visa avaliar os efeitos da interrupção do comportamento sedentário e do destreino na resposta pós-prandial da glicémia, na sensibilidade à insulina e na flexibilidade metabólica em idosos treinados.

Para tal, ser-lhe-á solicitada a sua participação **durante um mês**, no qual será sujeito, com acompanhamento de pessoas especializadas (enfermeiro e médico). No final deste documento, é disponibilizada uma tabela que ilustra os vários procedimentos que serão necessários realizar e que se encontram descritos, em texto, de seguida:

A **avaliação inicial** inclui seis procedimentos, a realizar em dois dias distintos: DIA 1 - **DXA** (densitometria raio-x de dupla energia) para avaliação da composição corporal e **bioimpedância**; **avaliação da flexibilidade metabólica** (medição do metabolismo de repouso durante meia hora com recolha de sangue, ingestão de uma refeição padrão; medição do metabolismo de repouso durante 2 horas, com recolha de sangue aos 60 e 120 minutos); DIA 2 - **prova de esforço** em passadeira para avaliação da aptidão cardiorespiratória; **avaliação da força muscular** (testes com sensores de força); preenchimento de **questionários** relativos aos hábitos alimentares, qualidade de sono, hábitos de atividade física e estado de saúde geral;

No **primeiro dia de intervenção, após os procedimentos da avaliação inicial** (DIA 3) será realizado um teste de tolerância oral à glucose (OGTT-2h) com recolha sanguínea em jejum e aos 120 minutos. Seguidamente, serão administradas duas refeições padrão (pequeno – almoço e almoço) e aplicado aleatoriamente um dos protocolos:

- **Protocolo A** consiste na manutenção de um comportamento sedentário durante 7 horas (3 horas durante a manhã + 4 horas durante a tarde);
- **Protocolo B** serão feitas interrupções de 2 minutos a cada meia hora no comportamento sedentário (3 horas durante a manhã + 4 horas durante a tarde). As interrupções consistirão na realização de um exercício, de dois possíveis: sentar e levantar da cadeira ou descer e subir escadas. Os exercícios serão realizados alternadamente entre os diferentes períodos de

interrupção do comportamento sedentário e a ordem de realização dos mesmos será definida pelas alunas que acompanham o protocolo.

No dia seguinte à aplicação de um protocolo (DIA 4) será realizado um OGTT (de manhã).

Após 4 dias da realização do primeiro protocolo, será aplicado o protocolo A ou B (exemplo: se no primeiro momento realizou o A, 4 dias após irá realizar o B).

Uma vez realizados ambos os protocolos, será sujeito a um **período de inatividade física de 15 dias**. Durante este tempo, solicita-se que não frequente as sessões de exercício que normalmente realiza na Faculdade de Motricidade Humana e fora desta, e que minimize os níveis de atividade física. Simultaneamente, ser-lhe-á solicitado o uso do acelerómetro em dois dias úteis e um dia de fim-de-semana, em cada uma dessas duas semanas.

Posteriormente, serão repetidos os procedimentos A e B, conforme o acima descrito, para comparar a resposta a vários parâmetros, após duas semanas de destreino.

A **avaliação final** consistirá na realização dos procedimentos feitos na avaliação inicial e será realizada após a repetição de ambos os protocolos.

A sua participação é voluntária e pode recusar-se a participar. Caso decida participar neste estudo é importante ter conhecimento que pode desistir a qualquer momento, sem qualquer tipo de consequência para si. No caso de decidir abandonar o estudo, a sua relação com a Faculdade de Motricidade Humana (FMH) não será afetada. Se for o caso, o seu estatuto enquanto estudante ou funcionário da FMH será mantido e não sofrerá nenhuma consequência da sua não-participação ou desistência.

Através deste estudo terá disponível, para seu conhecimento, informação detalhada relativa à sua condição cardiorrespiratória, composição corporal, valores glicémicos, sensibilidade à insulina, valores lipídicos e saúde metabólica. Adicionalmente, será informado/a de estratégias para interrupção do comportamento sedentário. Porém, este estudo não está isento de riscos, nomeadamente durante a prova de esforço, ainda que os mesmos sejam reduzidos devido à presença de um médico. Para além disso, poderá sentir algum desconforto decorrente das diversas colheitas de sangue, pelo que será aplicado um creme analgésico durante o período da recolha e ser-lhe-á fornecido um creme para colocar nos dias seguintes de forma a evitar o aparecimento de hematomas, como por exemplo nódoas negras.

Todos os dados deste estudo serão recolhidos, tratados e guardados (na FMH-UL e na Associação Protetora dos Diabéticos de Portugal) em regime de confidencialidade. Serão guardadas amostras de sangue suas, devidamente codificadas (garantido a confidencialidade dos dados), na FMH-UL destinando-se meramente para efeitos de investigação. Os resultados do estudo serão divulgados nas dissertações finais das alunas de mestrado e os mesmos ser-lhe-ão disponibilizados.

Em caso de dúvida ou situação de urgência, deverá ser contactada uma das alunas: Inês Correia (926149130), Júlia Lopes (916951976), ou Sofia Freitas (910357965).

Li (ou alguém leu para mim) o presente documento e estou consciente do que esperar quanto à minha participação no estudo: **Efeitos da Interrupção do Comportamento Sedentário e do Destreino na Resposta Pós-prandial da Glicémia, na Sensibilidade à Insulina, na Flexibilidade Metabólica e no Ângulo de Fase em Idosos Treinados.** Tive a oportunidade de colocar todas as questões e as respostas

esclareceram todas as minhas dúvidas. Assim, aceito voluntariamente participar neste estudo. Foi-me dada uma cópia deste documento.

Desde já, agradecemos o facto de se disponibilizar a ler este consentimento e, se assim o decidir, a participar no nosso projeto. Obrigado pela sua atenção!

Calendarização individual (calendarização tipo a adaptar para cada participante)

Dia	Horas	Local	O que fazer	Observações
8 Maio	A definir	Pavilhão Lord - FMH	Avaliação inicial	Equipado com fato de treino e ténis
12 e 13 Maio	**Não realizar AF vigorosa; no entanto, pode fazer caminhada leve; EVITAR CONSUMO DE BEBIDAS ALCÓOLICAS E BEBIDAS COM CAFEÍNA**			
14 Maio	7:30 – 10:30	Pavilhão Lord - FMH	Avaliação inicial	Em jejum e sem metais (ex: brincos)
14 e 15 Maio	**Não realizar AF vigorosa; no entanto, pode fazer caminhada leve; EVITAR CONSUMO DE BEBIDAS ALCÓOLICAS E BEBIDAS COM CAFEÍNA**			
16 Maio	8:00 – 18:00	Pavilhão Lord - FMH	OGTT + Protocolo 1	Vir em jejum
17 Maio	8:00 – 10:00	Pavilhão Lord - FMH	OGTT	Vir em jejum
19 e 20 Maio	**Não realizar AF vigorosa; no entanto, pode fazer caminhada leve; EVITAR CONSUMO DE BEBIDAS ALCÓOLICAS E BEBIDAS COM CAFEÍNA**			
21 Maio	8:00 – 18:00	Pavilhão Lord - FMH	OGTT + Protocolo 2	Vir em jejum
22 Maio	8:00 – 10:00	Pavilhão Lord - FMH	OGTT	Vir em jejum
23 Maio a 5 Junho	**DESTREINO:** Não realizar aulas estruturadas de exercício (ginásio ou piscina); **minimizar** atividade física (caminhadas, por exemplo); usar acelerómetro todos os dias; nos **dias 4 e 5 de Junho → EVITAR CONSUMO DE BEBIDAS ALCÓOLICAS E BEBIDAS COM CAFEÍNA**			
6 Junho	8:00 – 18:00	Pavilhão Lord - FMH	OGTT + Protocolo 1	Vir em jejum
7 Junho	8:00 – 10:00	Pavilhão Lord - FMH	OGTT	Vir em jejum
9 e 10 Junho	**Não realizar AF vigorosa; no entanto, pode fazer caminhada leve; EVITAR CONSUMO DE BEBIDAS ALCÓOLICAS E BEBIDAS COM CAFEÍNA**			
11 Junho	8:00 – 18:00	Pavilhão Lord - FMH	OGTT + Protocolo 2	Vir em jejum
12 Junho	8:00 – 10:00	Pavilhão Lord - FMH	OGTT	Vir em jejum
13 Junho	**Não realizar AF vigorosa; no entanto, pode fazer caminhada leve; EVITAR CONSUMO DE BEBIDAS ALCÓOLICAS E BEBIDAS COM CAFEÍNA**			
14 Junho	7:30 – 10:30	Pavilhão Lord - FMH	Avaliação Final	Em jejum e sem metais (ex: brincos)
20 Junho	A definir	Pavilhão Lord - FMH	Avaliação Final	Equipado com fato de treino e ténis

OGTT – teste de tolerância oral à glucose (tempo de realização 2h com duas colheitas de sangue)

Protocolo 1 e 2 – um dia sentado + um dia sentado com interrupções (10:30h – 18:00h)

Avaliação inicial e final

- **Prova de esforço** (avaliação da capacidade cardiorrespiratória)
- **Avaliação da força muscular**
- **Densitometria óssea** (avaliação da composição corporal)
- **Bioimpedância** (avaliação da composição corporal)
- **Saúde metabólica (com recolha de sangue)**
- **Questionários**

Assinatura do Consentimento Informado, Livre e Esclarecido

Li (ou alguém leu para mim) o presente documento e estou consciente do que esperar quanto à minha participação no estudo **Efeitos da Interrupção do Comportamento Sedentário e do Destreino na Resposta Pós-prandial da Glicémia, na Sensibilidade à Insulina, na Flexibilidade Metabólica e no Ângulo de Fase em Idosos Treinados**. Tive a oportunidade de colocar todas as questões e as respostas esclareceram todas as minhas dúvidas. Assim, aceito voluntariamente participar neste estudo. Foi-me dada uma cópia deste documento.

Nome do participante	**Assinatura do participante**

Data

Investigador/Equipa de Investigação

Os aspetos mais importantes deste estudo foram explicados ao participante ou ao seu representante, antes de solicitar a sua assinatura. Uma cópia deste documento ser-lhe-á fornecida.

Nome da pessoa que obtém o consentimento	**Assinatura da pessoa que obtém o consentimento**

Data